INTERCONVERSION OF CORTISOL AND CORTISONE IN NORMAL CONNECTIVE TISSUE OF MICE AND IN MALIGNANT (SARCOMA 37) CELLS IN HAMSTERS

by

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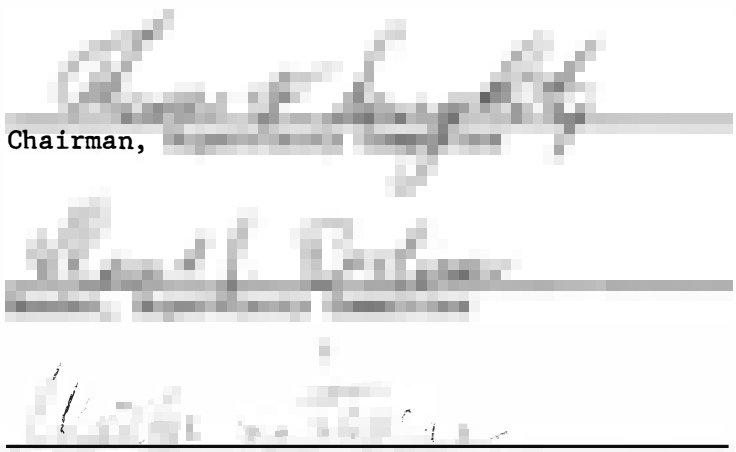

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INTERCONVERSION OF CORTISOL AND CORTISONE IN NORMAL CONNECTIVE TISSUE OF MICE AND IN MALIGNANT (SARCOMA 37) CELLS IN HAMSTERS

INTRODUCTION

The most important function of connective tissue appears to be the maintenance of the physiologic state. Fibroblasts constitute about 90 percent of connective tissue cells, and thus the state or activity of the fibroblasts and all factors which modify this state must be considered in relation to connective tissue functions.

The activity of fibroblasts is directly related to the maintenance of a suitable environment in the tissues for optimal cellular function; the physiologic state. Fibroblasts are the cells which produce or synthesize collagen fibers and are responsible for the turn-over of ground substance mucopolysaccharides. Ground substance itself, on the other hand, is the environment of these cells, and the state of the ground substance in turn modifies fibroblast activity.

Cortisol (Compound F) and cortisone (Compound E) are instrumental in maintaining the physiologic state following stressors like injury and subsequent inflammation. These hormones act directly on fibroblasts which are their major target cells in connective tissue.

Study of malignant cells in comparison to that of normal cells in connective tissue may yield information concerning the change or series of changes of the connective tissue as a whole or of a part thereof that may lead to malignancy.

In order to arrive at a better understanding of malignant cells, and so look forward to ways in which they may be controlled, experiments to be described have yielded data concerning the interconversion of labeled cortisol and cortisone in both normal connective tissue of mice and in Sarcoma 37 cells of hamsters.

This thesis, then, describes a study of malignant cells in comparison to that of normal cells in connective tissue with the hope of obtaining answers concerning the change or series of changes of the connective tissue as a whole or of a part that may lead to malignancy.

HISTORY

I. General History of Connective Tissue

Connective tissue is a special tissue composed of cells separated from one another by acellular matrix. It is derived from mesenchyme and thus from mesoderm. The elements of connective tissue are: (A) Ground substance, (B) cellular elements, and (C) fibrous elements. The functions are: (1) To support and surround the elements of other tissues, (2) to fill out the spaces between the organs and act as a packing material, (3) to play a role in the nutrition of the elements embedded in it, and (4) to play a role in inflammation (1,2,3,4).

Such early histologists as Jordan (1834), Henle (1841), and Burggraeve (1843), considered fibers the most important constituents in adult animal connective tissue, but they also described a few bare nuclei to which they paid little attention. Virchow (1859) recognized that the greater part of the connective tissue was composed of intercellular substance of varying chemical nature in which cells were embedded. He was the first to claim that these cells persisted in adult connective tissue, and also that the cells were quite similar in different types of connective tissue.

V. Recklinghausen (1863) was the first to describe two types of cells in connective tissue, one amoeboid and the other not; and he raised the question of the relationship between the two. He also said that in inflammation the pus cells were probably derived from white

blood cells rather than from connective tissue cells as Virchow contended. The studies of Ehrlich (1877-1879) showed that some of the connective tissue cells had a marked affinity for certain aniline dyes such as dahlia. These elements he called "Mastzellen" because he thought that they were a depot of reserve food material. Ehrlich also demonstrated that certain connective tissue cells contain granules which stained red with eosin. Thus, he distinguished three cell types in connective tissue; mast cells, eosinophils, and cells not stainable with either eosin or dahlia.

Before 1890, it was not customary to separate the outstretched spindle or star-shaped cells of the connective tissue into more than one type which were called connective tissue cells or fibroblasts or fibrocytes. However, Ranvier (1890, 1900) described another group of outstretched connective tissue cells which he called clasmatocytes, since he thought that they had the property of breaking loose small parts of their substance. He believed that the mast cells of Ehrlich were a variety of clasmatocytes. However, according to Maximow (1906, 1913) and Jolly (1923) clasmatocytes and mast cells could be distinctly separated in most animals. Therefore, at this time there was much confusion about macrophages (clasmatocytes), as we know them today, and their relation to mast cells and other cellular elements. Metchnikoff (1884, 1892, 1901), in a long series of investigations, called attention to the marked phagocytic properties of some of the connective tissue cells and called them macrophages. He ascribed to them an important

role in the "defense" reactions of the organism. His cytological and morphological work was inadequate however.

Thus by 1900 it was known that the connective tissue was composed of intercellular substance containing several cell types including mast cells, eosinophils, and fibroblasts or fibrocytes; and there was some confusion over clasmatocytes or macrophages.

Maximow (1902, 1903, 1904, 1905, 1906) not only studied the detailed cytology of different types of connective tissue cells but also the parts each played in inflammatory reactions. With supravital neutral red staining, he was able to distinguish between fibroblasts, macrophages (which he called resting wandering cells), ameboid wandering cells, connective tissue mast cells, eosinophils, plasma cells, and fat cells. Following connective tissue studies, Renaut (1904, 1907) called macrophages "Cellules rhagiocrines" and gave a secretory function to them.

The greater use of dyes, as acid aniline dyes and lithium carmine, led to a great extension of knowledge about the phagocytes of connective tissue (5). Histology texts as Bailey, Maximow and Bloom, and Ham give a clear picture of the development of connective tissue from stellate mesenchymal cells to macrophages, mast cells, fibroblasts, and endothelial cells. In 1940 Stearn (6) demonstrated that in the transparent rabbit ear chamber, fibroblasts are essential for the production of collagen fibers. Gersh and Catchpole (1949), R. E. Mancine (1950), Dougherty (1956), and Riley (1961) believe that the

ground substance in the adult is secreted by fibroblasts, while Asboe-Hansen (1951) says it is secreted by mast cells. Work by Dougherty and Higginbotham (7,8,9) from 1955 to 1957 has confirmed that shed mast cell granules are phagocytized by fibroblasts, and in 1958, Dougherty and Schneebeli (33) made pictures of this process. Following the work of Dougherty and Higginbotham, Riley (10,11) in 1961 and 1963 hypothesized that fibroblasts produce mucopolysaccharides which are secreted to the tissue spaces where they are broken down and later picked up by mast cells. These mast cells store the mucopolysaccharides until trauma and the ensuing local inflammatory response cause their release. Fibroblasts then complete the cycle by ingesting and digesting these mucopolysaccharides to satisfy their own metabolic needs.

II. Amorphous Ground Substance of Connective Tissue

The ground substance has been described as the amorphous continuum separating cells, vessels, and fibers of connective tissues. It consists of mucopolysaccharides, proteins, salts, and water (12,13).

A. Mucopolysaccharides

The mucopolysaccharides have received a great deal of attention. They generally contain a small but significant proportion of associated protein material with the complex polysaccharide part of the structure. Acid mucopolysaccharides are macromolecular polymers of hexoseamine and hexuronic acid. Their ionic properties arise from their uronic acid components and varying amounts of esterification with sulfuric acid

(Higginbotham, 14). They are also flexible chain polymers of high negative charge with a strong affinity for cations and water molecules. The following acid mucopolysaccharides have been isolated in connective tissue: Hyaluronic acid, chondroitin sulfate A, chondroitin sulfate B, chondroitin sulfate D, chondroitin, and keratosulfate (Meyer, 15). These acidic mucopolysaccharides exist in the tissues as complexes with other naturally occurring substances. They give the ground substance its gelatinous character, capacity to bind water, and physiochemical properties important to normal connective tissue function (Asboe-Hansen, 16). Ground substance varies from a liquid to a gel depending upon the state of polymerization.

B. Staining Characteristics

Ground substance is demonstrated by oxidative methods and metachromatic staining (Angevine, 17). The Hotchkiss or Periodic Acid Schiff (PAS) reaction probably indicates the presence of polysaccharides (Robb-Smith, 18). Under certain conditions, the ground substance stains metachromatically. (Metachromasia is the phenomenom by which certain tissue structures are stained by basic dyes as toluidine blue in a color different from the color of the dye solution.) Gersh's theory (19) of the structure of ground substance holds that ground substance, if highly polymerized, stains weakly with Hotchkiss procedure, is palely stained with Evans blue, will show little or no metachromasia, and tends to be in a gel-form. Partially depolymerized

ground substance stains more strongly with Hotchkiss procedure, has a marked affinity for Evans blue and may be more metachromatic. Finally, highly depolymerized ground substance may stain more or less strongly with Hotchkiss procedure, will have a marked affinity for Evans blue, will probably be more metachromatic, and will in the end become water soluble. At this point, it may be broken down into particles small enough to enter the blood as mucoproteins (20,21,19). Since changes in ground substance include increase or decrease of metachromatic staining, frequently accompanied by changes in the intensity of staining by oxidative method, these changes may represent the degree of polymerization, changes in viscosity, or changes in the degree of sulfation (McManus, 23).

C. Origin of Ground Substance

There are two schools of thought as to the origin or source of the mucopolysaccharides found in ground substance. One school, which includes Gersh and Catchpole (19,20,21) believes that the fibroblasts produce and secrete the material which forms ground substance. They suggest that the fibroblast may control the state of polymerization of the ground substance and that the precursor of ground substance is a glycoprotein in the fibroblast together with a carbohydrate radical. They have also shown that granules and vacuoles in fibroblasts give the histochemical reactions of mucopolysaccharides, and that there are large amounts of mucopolysaccharides in certain types of fibrosarcoma.

The second school, led by Asboe-Hansen (16) believes that the mast cell is the source of connective tissue mucopolysaccharides. Among other evidence, he points out that the mast cell stores, if not synthesizes heparin, a non-acetylated sulfuric ester of glucuronic acid.

It is not inconceivable that the ground substance mucopolysaccharides come from both of these sources.

III. The Fibroblast in Connective Tissue

A. Description and Histology

Fibroblasts are multipotential cells which are separated by ground substance and make up 90 percent of the cellular elements of loose connective tissue. They are called fibroblasts and not fibrocytes to denote their ability to form intercellular fibers and to change into other cell types when needed. According to most investigators, these cells occur only in loose and dense connective tissues (3).

In living loose connective tissue, the fibroblasts are sometimes difficult to observe as they are obscured by collagenous fibers (3). Their star-shaped bodies usually send out extensive cytoplasmic processes which may branch and anastomose with those of other fibroblasts. These cell bodies may be demonstrated with iron hematoxylin. The nucleus is generally large, oval and stains lightly. It contains one or more round or angular pale staining nucleoli, and dust-like chromatin particles which are distributed quite uniformly throughout the nucleus.

The cytoplasm normally stains very lightly and is homogenous or finely granular. Numerous mitochondria, appearing as slender rods, are found closely packed, especially near the nucleus (Meves, 1910; Maximow, 1916, 1928). The cytoplasm also contains a diplosome and a Golgi net near the nucleus. Fibroblasts normally contain few inclusions except an occasional vacuole or fat droplet. Inclusions are found in tissue culture, inflammation, or upon injection of foreign particles or macromolecules (3,4,5,24,25).

McManus (23) states that fibroblasts may contain inclusions of fat or protein and commonly contain carbohydrate. He also states that carbohydrates and lipids occur in the classical position of the Golgi element. Gersh and Catchpole (1949) have described the presence of PAS positive granules in the cytoplasm of fibroblasts and have related this to alterations in the PAS positivity of ground substance. With marked cellular hyperactivity, PAS positive granules are not present (23). Cytoplasmic basophilia is said to occur during increased cellular activity (repair, tumor) and is related to metabolic activity. There also appears to be some relationship between the granulation and frequency of mast cells and the basophilia and activity of the fibroblast.

The use of acid aniline dyes and lithium carmine in vital staining permits a ready means of separating fibroblasts from macrophages. Following i.v. or i.p. injection of these dyes, macrophages are found to store large amounts, while fibroblasts contain only a few granules.

Large amounts of trypan or isamine blue are also stored by fibroblasts, but generally in fewer and smaller granules. Fibroblasts have been shown to store these particles for a much longer period than do the macrophages. Even in extreme dye storage, the two cell types are distinguishable by their individual cytological characteristics; different nuclei, larger size of fibroblasts, etc. (Bloom, 5).

Each fibroblast acts as an independent cellular element. In inflamed tissue and in tissue culture it moves with a gliding motion. Pseudopodia are usually not observed, but cells following in the direction of pinocytic filaments have been filmed (Schneebeli, 26). In general, fibroblasts are not phagocytic for most things, but they do take up mucopolysaccharides, India Ink, and glucan, and may store some of them for extended periods.

Fibroblasts are scattered singly or in groups among the collagenous fibers in the loose connective tissue. Some workers as Dominici (1920), Heringa (1924), and W. and M. V. Mollendorff (1926) believe the cytoplasm of adjacent fibroblasts to be continuous and form a syncytium; whereas W. Lewis (1922, 1926), Levi (1923), and Maximow (1916, 1927) believe that the processes are often in contact, but do not form a true syncytium. This latter opinion is generally accepted now (5).

In late stages of scar formation, in inflammation, and tissue culture, and frequently about invading carcinomas, long thin fibrils have been described in the cytoplasm of the fibroblast. Maximow

first described them in 1902 in iron-hematoxylin stained preparations and called them tono-fibrils due to their resemblance to similar fibrils in other cells. In 1904, Mallory demonstrated them also, calling them fibroglia fibers. They have sometimes been considered as precursors of the intercellular connective tissue fibers (5).

B. Origin and Developmental Potencies of Fibroblasts

Fibroblasts are direct descendents of the mesenchymal cells of the embryo, have a similar appearance to them, and it is difficult to tell at what particular stage a mesenchyme cell becomes a fibroblast. At any rate, fibroblasts gradually develop by individual metamorphosis of individual embryonal cells.

There is still some difference of opinion as to whether the fibroblasts of loose connective tissue are undifferentiated elements or whether they are an end type cell. V. Mollendorff (1926), Weidenreich (1911), Benninghoff (1926), and others believed that the fibroblast of adult connective tissue is endowed with all the properties of mesenchymal cells, while Maximow (1902, 1927), Marchand (1924), and Aschoff (1924) agreed that fibroblasts of adult connective tissue are more highly differentiated cells. However, Maximow (1907) and Weidenreich (1930) felt that there was no doubt that in abnormal conditions fibroblasts could develop into osteoblasts and osteocytes (Bloom, 5).

From tissue culture experiments much controversy arose as to whether adult fibroblasts could change to macrophages or macrophage-like cells, and/or vice versa, thus proving developmental potencies.

From subcutaneous tissue cultures of the adult rabbit, Maximow (1916) concluded that fibroblasts are not converted into amoeboid cells in vitro, but that some macrophages can become fibroblasts in vitro. He believed that transitional forms between histiocytes and fibroblasts (seen in vitally stained animals) indicated development of fibroblasts from macrophages. M. von Mollendorff (1929), working also with rabbits, determined that fibroblasts of adult connective tissue can develop into macrophages, and showed in 1932 that fibroblasts would phagocytose or take up large quantities of carbon and R.B.C. On the other hand, Bloom (1931), who repeated these experiments, concluded that fibroblasts could turn into macrophage-like cells, but emphasized that the phagocytic nature of these cells had not been proved. There is now strong evidence that the rounding up of fibroblasts, resulting in resemblance to macrophage-like cells, is a property of the fibroblast in response to stress or application of hormones such as cortisol or cortisone (Dougherty, et al, 27,28,29). There is also evidence that some of the lymphocytes which enter the connective tissue from the blood during inflammation may turn into macrophages. As the inflammatory process dies down, these macrophages may settle down as resting macrophages and some may later become fibroblasts (Bloom and Fawcett, 3).

Another old idea of Virchow (1859) was revived by V. Mollendorff (1926) to the effect that in inflamed tissues exudate cells develop from fibroblasts after passing through a histiocyte stage. Maximow (1927, 1929) and Bloom (1928, 1931) felt that PMN's, lymphocytes and

monocytes came from the blood stream and that fibroblasts could be but an exceedingly insignificant source of polyblasts even in inflamed tissues. This is because of their small numbers as compared with lymphocytes. Maximow (1902) believed that lymphocytes may develop into fibroblasts (Bloom, 5). There is little doubt of this now.

After these early controversies, the ability of the common fibroblast of the connective tissue to develop into macrophages and other types of blood cells was neither completely proved nor disproved, but it was considered definite that certain cells in adult connective tissue do have mesenchymal properties. Original mesenchymal potencies are retained by cells (undifferentiated mesenchymal cells or perivascular cells) scattered in the loose connective tissue along the blood vessels. They are believed to be fibroblasts, endothelial cells, or fixed macrophages (Bloom and Fawcett, 3). These cells were demonstrated to turn into free macrophages, lymphocytes and myelocytes (Marchand, 1924; Maximow, 1926); and into osteoblasts and fat cells (Herzog, 1928, and Bloom, 5).

At present, the majority opinion holds that fibroblasts are differentiated cells which generally do not give rise to other types of free cells in the loose connective tissue (Bloom and Fawcett, 3). Some investigators believe that, under the influence of external stimuli, all fibroblasts can produce any cell type of the blood and connective tissue (Bloom and Fawcett, 3). There is, however, an increasing opinion that the fibroblast is a multipotential cell,

which under certain conditions may become an endothelial cell, a fat cell, a chondroblast, an osteoblast, etc.; but that it probably does not give rise to macrophages and only rarely forms myeloid elements (Bloom, 5; Dougherty, 24).

There is also much feeling that fibroblasts, lymphocytes, and RE cells are closely related functionally, and there is evidence that they may be morphologically and functionally interchangeable under certain circumstances (Dougherty, 24).

C. Functions of Fibroblasts

With changes in function, the morphology of fibroblasts may change. Some fibroblasts in a population may be specialized for the formation of collagen, others for polysaccharide, some for deposition of fat, etc. Steroid hormones may also influence the functions of these look-alike cells.

1. Synthesis of Materials and Structures: Fibroblasts perform most of the functions attributed to the supporting framework of the body. They construct much of their framework and produce the matrix in which they find themselves (Dougherty, et al, 27; Berliner and Dougherty, 30). Fibroblasts are essential for the production of collagen fibers, may produce elastic fibers, and may synthesize or produce ground substance mucopolysaccharides and participate in the metabolism of these tissue mucopolysaccharides (Dougherty and Berliner, 28). Fibroblasts also synthesize as well catabolize mucoproteins (Higginbotham, 14). Also, they are major producers of cholesterol

and may secrete this substance (Dougherty and Berliner, 29,31).

2. Localization of Cortisol: That the fibroblast is the main cellular site for the localization of cortisol was shown by Dougherty and Schneebeli (27). They determined the cellular localization of cortisol in connective tissue by giving C^{14} labeled cortisol subcutaneously to adrenalectomized mice. It was found that the fibroblasts contained the hormone for a time either in the cytoplasm or at the cell surface. At any rate, cortisol produced definite changes in the appearance and function of fibroblasts during the time it acted on these cells. Macrophages and lymphocytes did not appear to concentrate cortisol or retain it for any long period, and they could not perform the transformations that fibroblasts do. Fat cells also contained little cortisol. Thus, the greatest amount of radioactive hormone was concentrated by fibroblasts.

D. The Action of Cortisol on Fibroblasts

All actions of cortisol on fibroblasts are direct effects and do not appear to be mediated through systemic mechanisms. The fact that one event may be affected by a different dosage than another event is important in the cause and treatment of connective tissue disease (Dougherty, et al, 28).

1. Interference with Fibroblastic Functions: The proliferation of fibroblasts or their multiplication may be inhibited by cortisol (Dougherty, et al, 28,29). Collagen deposition likewise may be reduced by cortisol. Since cortisol tends to inhibit wound healing

and growth of fibroblasts in tissue culture (Dougherty and Schneebeli, 32), it may act by interrupting mitosis. Cortisol may also decrease the synthesis of ground substance, since it has been shown that synthesis and/or deposition of polysaccharides is inhibited by cortisol (Dougherty, et al, 28,29,33).

2. Inflammation: The most important physiological action of cortisol on the fibroblast occurs during inflammation (Dougherty, 34,35). Inflammation, actually, is just a pathological expression of normal physiological processes.

At an early stage of the inflammatory response, cortisol interrupts and inhibits the chain reaction of fibroblastic destruction by causing the fibroblasts to pull in their processes, round up, and become epithelioid in appearance (Dougherty, 29,35,36). Cortisol exerts this antiphlogistic effect by inhibiting the focal damage to fibroblasts and by preventing the influx of PMN's, lymphocytes, and macrophages into the injured area (Ragan, 37). The rounded fibroblasts may persist in spite of enormous fibroblastic damage. The severity of the inflammation and the amount of cortisol present determines the amount of fibroblast destruction. However, the less the metabolism of cortisol, the greater its anti-inflammatory effect, and vice versa (31,35).

It has been found in our laboratory (33,38) that the length of time that cortisol is present in the fibroblast is much shorter than the period in which inflammation is reduced. The maximum concentration

of radioactive cortisol in the connective tissue after administration was found to be about 40 minutes, whereas the anti-inflammatory effect lasts about three hours and sometimes more. It therefore appears that cortisol triggers some mechanism which continues after the hormone is catabolized and excreted (38). It has also been demonstrated that though there are no special mechanisms tending to localize cortisol in connective tissue in inflammation, more cortisol is found in the inflammatory area due to the edema which brings in many non-specific substances, including cortisol.

The 17 OH structure on the steroid nucleus is absolutely necessary for the anti-inflammatory effect and for the epitheliazation of fibroblasts, whereas the OH at position 11 is not necessary. The most effective compounds in this respect include cortisol, which is the most effective, cortisone, and Substance S.

Conversion of cortisol to other compounds is inhibited in inflamed connective tissue. In other words, fibroblasts in the zone of inflammation seem to have lost their ability to oxidize or reduce the positions of the cortisol molecule. However, it may be that in incubations of inflamed connective tissue, where there are fewer fibroblasts due to many having been destroyed, steroid compounds, if present, could not be detected by the methods used (28,29,32,33,38,36, 35,39,40).

Thus, inflammation once initiated could be greatly affected by a deficiency of the hormone supply or by alteration in fibroblast metabolism.

3. Destruction of Fibroblasts: Large doses of cortisol destroy fibroblasts and RE cells. Smaller doses of cortisol cause destruction of some cells and change the remaining cells as discussed above. Pinocytosis is absent and the fibroblast appears anesthetized. The cytoplasm becomes densely basophilic and contains vacuoles which are filled with reducing substances as shown by the phenyl hydrazine, TPTZ, and tetrazolium blue reactions. Fibroblasts began to assume a spherical shape within 15 minutes after the tissues were exposed to cortisol. After 45 minutes, the hydrazine reaction could be shown by the presence of positive granules in the fibroblasts. After one hour, NTZ granulation was noted demonstrating the presence of reducing groups. These substances persisted after radioactive cortisol could not be detected (27,28,29,31,33).

IV. Effects of Hormones on Connective Tissue

Hormones exert regulatory influence on connective tissue cells (16,37,41,42,43). Although primary attention is directed to the effects of cortisol on fibroblasts, other hormones also exert influences. These will be reviewed here.

Hormones may accelerate or depress the activity of normal processes in connective tissue (Loeb, 37). They may interfere with 100 or more enzyme systems (Asboe-Hansen, 16,41). The hormones active in connective tissue metabolism are many. A summary of hormonal actions on connective tissue is presented in Table I by Taubenhaus (22).

First, the pituitary hormones will be discussed, including growth hormone, thyrotropic hormone, and gonadotropin. These hormones may act through their target organs, or directly as may be noted below. Then the other hormones, including adrenal cortical hormones, thyroid hormones, parathyroid hormones, the sex hormones (estrogens and androgens), and progesterone will be discussed. The effects of cortisol have already been reviewed separately in greater detail.

A. Growth Hormone

Growth hormone accelerates and increases fibroplasia, stimulates mast cell production of acid mucopolysaccharides, stimulates the formation of collagen from fibroblasts, and stimulates the proliferation of granulation tissue (Asboe-Hansen, 16; Ragan, 37).

B. Thyrotropic Hormone

This hormone has a direct effect upon the connective tissue. An injection of this hormone in guinea pigs is followed by a mobilization of fat from normal depots to liver, kidneys, cardiac, and skeletal muscle (Duran-Reynals, 43; Asboe-Hansen, 16,44). Tissue fat is replaced by a mucinous substance, mainly hyaluronic acid. The content of hyaluronic acid in connective tissue is increased in myxedemateous (hypothyroid) subjects, manifesting itself by an inhibition of spreading in the skin, but increasing the spreading effect of hyaluronidase. Thyrotropic hormone may affect the function of mast cells, perhaps increasing their number and influencing them to secrete hyaluronidase-

sensitive mucopolysaccharides as hyaluronic acid into the connective tissue (Ragan, 37; Asboe-Hansen and Iverson, 45). Since mucopolysaccharides, as hyaluronic acid, possess a marked capacity to bind water, an increased content of these substances may explain exophthalmos.

C. Gonadotropin

Gonadotropin increases the spread of tubercle bacilli in the skin of rabbits, increases vascular permeability, and increases the permeability of connective tissue ground substance as a whole (Asboe-Hansen, 16).

D. Adrenal Cortical Hormones

Adrenal cortical hormones as cortisol and cortisone decrease fibroplasia and have anti-hyaluronidase action, i.e., they reduce the spreading capacity of hyaluronidase in rabbit and human skin. They also depress or inhibit depolymerization by hyaluronidase (16,41,44, 46,47,48). These hormones, especially cortisol, are used in diseases of mesenchymal tissues, in hypersensitivity, in acute inflammation, and in some allergic diseases due to their anti-inflammatory activity (27,32,46,49,50). Desoxycorticosterone (DOCA) should be mentioned as its effects are opposite to those of cortisol and cortisone. It accelerates and increases fibroplasia (37), increases depolymerization of mucopolysaccharides by hyaluronidase, and counteracts the inhibitory effect of cortisone in wound healing (37). It also acts in the absence of the adrenals (Seifter, 47).

E. Thyroid Hormone

This hormone resolves myxedematous connective tissue changes, and causes the accumulation of mucinous substances, mainly hyaluronic acid, to subside (Asboe-Hansen, 16,41). However, it decreases the spreading effect of hyaluronidase since there is less hyaluronic acid to act on. Further, thyroid hormone inhibits pituitary production of thyrotropic hormone, increases the metabolic rate, causes the mast cells to become small and faintly granulated, and increases spreading in the skin.

F. Parathyroid Hormone

This hormone may increase the production of mucopolysaccharides and decrease the permeability of connective tissue (Asboe-Hansen, 16).

G. Estrogens

In loose connective tissue, estrogen is responsible for an increase in the immature elements. It induces a rejuvenation process. In the dense connective tissue, it stimulates maturation (Muller, 51). However, Meir, et al (52), have done in vitro experiments with estradiol in which disturbances in mitosis of fibroblasts were produced without essentially affecting the growth of fibroblasts.

Estrogenic hormones inhibit spreading in rabbit skin (Asboe-Hansen, 16). There is an increased resistance to the spread to tubercle bacilli, other infections, and India Ink. This may possibly be due to an increase in the mucopolysaccharide content of

the connective tissue, especially of hyaluronic acid. However, intradermal injection of hyaluronidase, when estradiol is locally applied to rabbit skin, increases the rate of spread in rabbit skin (Baker and Abrams, 42).

Old male fowls, caponized or treated with diethylstilbesterol showed a marked decrease in the tensile strength of the skin and muscles and a decrease in the amount of collagen in these tissues (Herrick and Brown, 53).

Naturally, the effects of estrogen are considerably modified by the ovaries, pituitary, and adrenals (Muller, 51).

The sex hormones could not be regarded as significant growth stimulating agents upon fibroblasts (Von Haam and Cappel, 54). It was shown, however, that estrin, at certain levels, did increase the mitotic index over controls and that the surface area of growth was increased.

H. Androgens

Testosterone propionate, given to capons, causes them to develop increased tensile strength and collagen levels in the skin, which decreases after this hormone is discontinued (Asboe-Hansen, 16; Herrick and Brown, 53). Applied locally in the normal animal, testosterone is said to inhibit the migration of WBC, and to inhibit the growth of fibroblasts to a slight extent (Meir, et al, 52). With androgen administration there may also be an increase in polysaccharides as hyaluronic acid in the cock's comb; and it may be that androgens increase the

synthesis of hyaluronic acid (52).

I. Progesterone

The action of progesterone appears to be somewhat opposite to that of estrone (Asboe-Hansen, 16). It appears to inhibit the migration of WBC and to inhibit the growth of fibroblasts to a greater extent than does testosterone (Meir, 52).

J. Aging

Aging should be mentioned in passing, since it certainly affects connective tissue considerably. With advancing age, the ratio of ground substance/collagen decreases and binding of water decreases. Thus, the formation of mucopolysaccharides in connective tissue is reduced, the mast cell count drops, the connective tissue macrophages are decreased, and elastic and collagen fibers change (Asboe-Hansen, 16).

V. Corticosteroids: The Adrenal Cortical Hormones

A. The Chemistry of Adrenal Cortical Hormones

Thirty crystalline compounds have now been obtained from extracts of adrenal glands. These steroids include estrone (C-18 compounds); some androgens (C-19 compounds); progesterone, cortisol, cortisone, etc. (C-21 compounds); and cholesterol (C-27 compounds). Of the adrenal steroids, seven have significant adrenal cortical activity (White, Handler and Smith, 55; Sayers, 56). See figure 1.

B. Relation of Structure to Physiological Activity

The physiological activity of corticosteroids varies with variations in chemical structure. Hydroxylations at key positions change the biological activity of a steroid. For example, progesterone has high gestagenic and mild mineralocorticoid effects. Hydroxylation of progesterone at C-21 gives desoxycorticosterone, a compound with more sodium-retaining activity. Hydroxylation of progesterone at C-21, and C-17 gives us cortisol which has gluconeogenic, anti-inflammatory, and lymphokaryorrhetic activities. Four structural features are of great importance in determining the biological activity of these compounds (Pincus, et al, 57): (1) The alpha-B unsaturated 3-ketone grouping; (2) the side chain with a ketone at C-20; (3) an alcoholic or ketonic oxygen atom substituted at C-11; and (4) the presence or absence of an OH at C-17. A C-11 OH regulates lymphatic tissue growth (58,59,60,61, 62,63), whereas a C-17 OH is necessary for anti-inflammatory effect and for epithelialization of the fibroblast (Dougherty, et al, 30).

C. Metabolism of Adrenal Cortical Hormones - Cortisol

In studies of laboratory animals and in normal and diseased humans, it has been found that there is a considerable similarity in the ways in which adrenal cortical hormones are metabolized. Cortisol is synthesized by adrenal cortical cells and released to the blood. It is carried in the blood bound to a specific globulin called transcortin, which is produced in the liver. The amount of transcortin is increased by ACTH and estrogen (55).

The metabolism of cortisol (F) is very rapid. The steroid has a half life of about 47 minutes in the normal human, and nearly the same in rats and mice. As soon as the hormone arrives in the blood, it begins to leave. It passes from the capillaries into the connective tissue in a passive manner, similar to that of other blood constituents. Following administration in the mouse, the maximum concentration of cortisol is found in the blood at 20 minutes, but the maximum concentration of hormone in the connective tissue is not reached until 40 minutes. Cortisol moves through the ground substance and tends to localize at the surface of the fibroblasts, or in them, causing them to round up and resemble epithelial cells. This process enables the cell to acquire resistance to the waves of cellular destruction occurring in inflammation by interrupting the chain reaction of cellular damage. This has been shown by histological, histochemical, and radioautographic studies in our laboratory (28,30,32,38,36).

Through a series of oxidations and reductions, cortisol is rapidly transformed or converted by the fibroblasts to other steroid hormones which are in general either inactive (substances E and U of Reichstein) or potentially active as cortisone. See figure 2. These end with removal of the side chain (28,29,31,59,64).

As the conversion products of cortisol are formed, they pass into the blood and to the liver. Here they are conjugated mainly with glucuronic acid and subsequently excreted by both the renal and biliary systems (28,30,36). Thus, cortisol can be changed chemically by the fibroblast in the connective tissue.

D. Biotransformation

Biotransformation of steroids is metabolism occurring by a series of oxidations and reductions in non-endocrine tissues. This is peripheral metabolism and it includes hepatic and extra-hepatic metabolism. It is transformation toward a compound having a different function than the original molecule (28,39).

One of the most active cells of the extra-hepatic metabolism of steroids is the fibroblast. It has the ability to oxidize and reduce the various substituted groups on the gonane nucleus and can thus regulate the degree of activity or inactivity of the steroid molecule (Dougherty and Berliner, 30; Dougherty, et al, 28). For example, the fibroblast may transform cortisol to steroids completely lacking in anti-inflammatory activity (Dougherty and Berliner, 31).

Metabolites isolated in our laboratory include cortisone, Reichstein's E and U, some dihydro F, and C-19 compounds as androstenedione. There is some corticosterone also (28, 31; Berliner and Dougherty, 64). Molecular changes include oxidation of the OH group in position C-11, some reduction of the 4-5 double bond, reduction of the C-20 ketone (C-20 ol form) and oxidative cleavage of the side chain (Dougherty, 28,36).

When cortisol is metabolized to cortisone, it may in part still be reversibly transformed to cortisol and thus be biologically active; but if it is metabolized to compounds reduced at the C-20 or those in which the side chain has been removed, it is irreversibly transformed

to biologically inactive compounds. Cortisol also has a slight ability to reduce ring A to give dihydro F (Berliner and Dougherty, 39). Thus, the degree of reversibility regulates the amount of biological activity. Also, in general, the greater the state of reduction of the steroid molecule, the less active it is biologically; and if biotransformation can be inhibited, biological activity can be enhanced (28,39). Co-factors DPN, TPN, DPNH and TPNH are necessary for the transformations of cortisol by fibroblasts.

TABLE I

HORMONE SYNERGISM AND ANTAGONISM

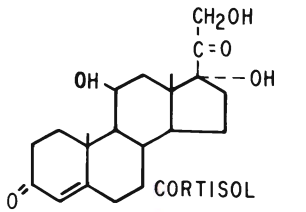
IN TISSUE REACTIONS

Taubenhaus, M. (22)

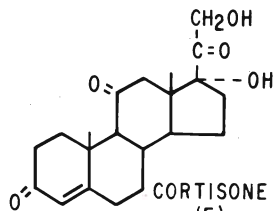
	General Effect	Fibro.	Collagen Fibers	Ground Substance
Ant. Pit. Growth Hormone	Stim.	Large, normal contour	Thick	?
Thyroxin	Stim. in deficient animals	Normal	Normal	Abolish mxyedema
DOC	Stim.	Large, poly-gonal or stellate	Dim.	Altered
Cortisone	Inhib.	Small	Inhib. of longitud. growth	Altered
Estrogen	Inhib.	Small, flat	Thin	Altered?
Testosterone	Inhib.	Small	Thin	Altered?

Footnote: Taubenhaus, M. Hormone Synergism and Antagonism in Tissue Reactions. Reprint from Bulletin de l'Academe Suisse des Sciences Medicales Vol. 8 (1952), Fasc. 1/2, p. 58

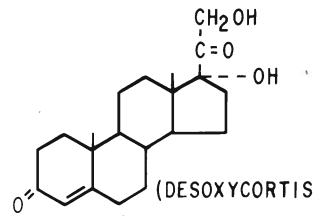
ADRENAL STEROIDS WITH SIGNIFICANT AC ACTIVITY



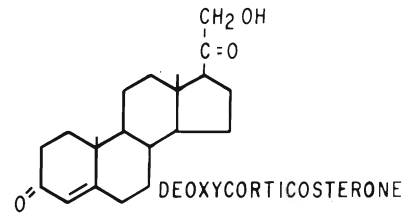
(F) HYDROCORTISONE
17α OH CORTICOSTERONE



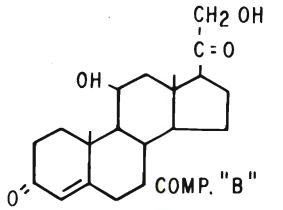
(E)
11-DEHYDRO-17α CORTICOSTERONE



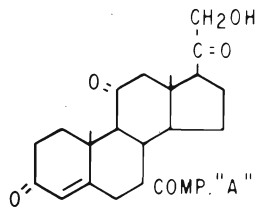
11-DEOXYCORTISOL
11-DEOXY-17α-OH CORTICOSTERONE



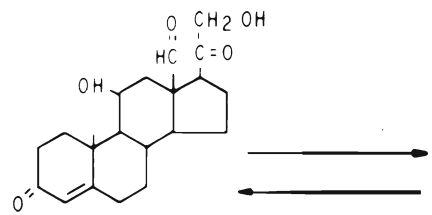
DOC
DESOXYCORTICOSTERONE



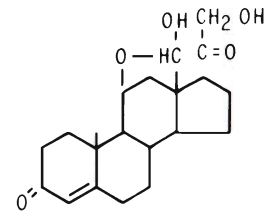
CORTICOSTERONE



11-DEHYDROCORTICOSTERONE



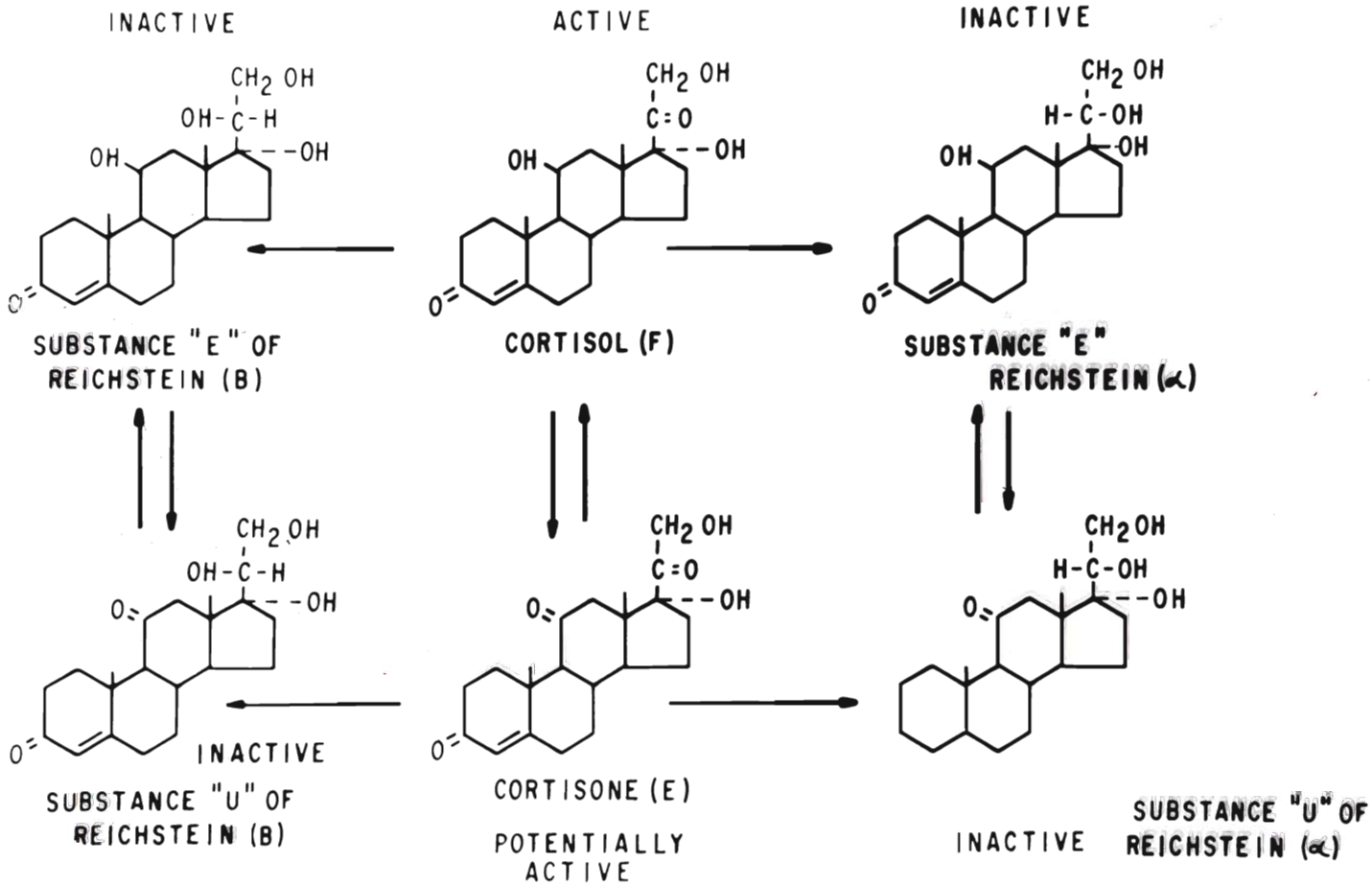
ALDOSTERONE



HEMIACETAL

ADRENAL STEROIDS WITH ADRENAL CORTICAL ACTIVITY

FIBROBLASTIC METABOLISM OF CORTISOL



(2)

VI. The Sarcoma 37 Cell

A sarcoma (fleshy tumor) is a malignant connective tissue neoplasm. Sarcoma 37 is a particular connective tissue tumor which has been transplanted consecutively over a long period of time. It is very malignant and may cause death in about 30 days when injected subcutaneously in hamsters. There are two forms of the tumor; the ascites form which is transplanted intraperitoneally, and the solid form which is transplanted subcutaneously or intramuscularly. This tumor is homotransplantable, though it shows a definite preference for some strains. Mice and hamsters appear to be the animals of choice. In rats it gives a percentage of takes, but the tumors usually regress (Pikovsli and Schlesinger, 65).

A. Microscopic Picture and Characteristics of Sarcoma 37

If ascites sarcoma cells are injected into peritoneal cavities of mice, most of the tumor cells may be harvested as a cell suspension in the peritoneal fluid. However, cells injected subcutaneously form a solid mass of adherent cells. The cells may change from the ascites form to the solid tumor form and vice versa, depending upon the site of injection. Cook, Seaman, and Weiss (66) compared seven day Sarcoma 37 ascites and solid tumor cells and showed by cell electrophoresis that there was a significant difference in mobility between cells derived from the ascites and solid forms of mouse Sarcoma 37.

1. Cell Types: Studies were performed on the solid form of

mouse Sarcoma 37 grown in hanging drop tissue culture, and the cell types were examined (Hogue and Rubin, 67). After 24 hours, three cell types were observed. (1) Spindle-shaped cells with blunt processes, (2) cells with irregular tortuous processes (looking like fern fronds) which apparently become spindle-cells after two or three days, and (3) small cells resembling mouse lymphocytes, which were amoeboid.

Death from intracavitary inoculation occurred in most animals earlier than that following subcutaneous inoculation. Subcutaneous tumors of the flank invaded connective tissue and muscles by several encircling layers, which in their growth obliterated normal tissue and vessels with perivascular damage and hemorrhage. The tumors reached considerable weight, 2-4 grams. Survival times of mice depend upon the route of injection. These are summarized in Table II (Goldie, et al, 68). Mortality of mice given inoculations by various routes and various topographical sites illustrates that there may be early death in spite of a small amount of tumor tissue or later death when the tumor is large and organized.

Chu (69) studied intramuscular Sarcoma 37 transplanted subcutaneously into CAF mice. He noted that before vascularization occurred at 3-4 days after inoculation, the cells were arranged in three zones: (1) Peripheral zone of giant cells, (2) an intermediate zone of spindle cells, and (3) a central zone of necrotic cells. After granulation tissue brought in more macrophages and giant cells and became incorporated into the implant, a new layer of granulation tissue formed around

the growing tumor, and this now well-vascularized tumor was observed to contain a mixture of spindle and necrotic cells. Chu noted that vascularization seemed to supply nutriment as well as cellular elements for growth of the daughter tumor.

Other authors, including Sylven (70,71) have described three areas in Sarcoma 37 tumors: (1) An outer layer of cells (A cells) containing the growing and dividing cell types, (2) an area of B cells near the center which are non-growing tumor cells with different cytological and enzymatic characteristics, and (3) a central necrotic area, the size depending upon whether the tumor is young and well-vascularized with a small necrotic area, or not.

2. Metabolism: Obviously, the composition of central fluids will be influenced by products released during cell injury and autolysis. In the B cell compartment relatively high levels of glucose were found (Burgess and Sylven, 70). This may suggest a deficient utilization of glucose. Also, lactate concentration was lower here than in young fully vascularized tumors.

Many studies have been done as to the characteristics of Sarcoma 37 tumors. Most tumor tissues, including Sarcoma 37, have a decreased content of glycogen together with a high rate of glycolysis (Nigram, et al, 72). These same tissues also showed a lower activity of phosphoglucomutase and glycogen synthetase, as compared with normal liver and muscle. There were also decreased phosphorylase levels. Nigram and co-workers (72) suggest that these tumors may contain a

defective system for glycogen synthesis owing to low activities of the enzymes involved.

Microscale assays of protein, glucose, lactate (LA) and total lactic dehydrogenase (LDH) activity in samples of free interstitial fluid from solid unicentric transplants in mice (intramuscular Sarcoma 37 tumor) were compared with those in plasma and intraperitoneal fluid from normal and tumor mice (70). The tumor fluids contained greater amounts of protein, LA, LDH, and glucose than normal interstitial fluid - the highest values being in the central fluid of young tumors. The authors concluded that the composition of interstitial fluid of solid tumors is very different from that of normal interstitial fluid. It was also pointed out that there are marked differences between Sarcoma 37 solid and ascites tumors. For example, it is considered that there is a high rate of glucose metabolism in many solid tumors (including Sarcoma 37), and therefore, one might expect low glucose levels in these tumors. However, there are found to be large quantities of glucose in both the peripheral and central fluids of these tumors while ascites tumors contain low levels of glucose. Also, there appear to be lower enzymatic activities (arginase, dipeptidase, glutathione reductase) in older tumor cell generations than in young ones and in ascites tumors than in solid ones (Malmgren and Sylven, 71,73).

When soluble proteins from different types of malignant tissues in mice were chromatographed on DEAE (diethylaminoethyl cellulose),

Sarcoma 37 and all other tumors examined showed a similar protein and enzyme pattern (Pikovsli and Schlesinger, 65; Roberts, 74). The authors felt that the results indicated that tumor proteins resemble one another closely and that tumors, regardless of their tissue of origin, have similar enzymatic patterns.

There was also a consistent pattern of distribution of free amino acids in malignant tissues which was different from that of any of the normal tissues studied (Angeletti, et al, 75).

Daoust and Amano (76) examined the ribonuclease and deoxyribonuclease activity of 65 experimental and human tumors (including Sarcoma 37) by histochemical substrate film methods. The same general pattern for the distribution of these nucleases was found. The connective tissue stroma and necrotic regions of the tumor masses showed various levels of nuclease activity, whereas the neoplastic cells showed none. It appears that deficiencies in the two nuclease activities represent general properties of cancer cells. The authors suggested that loss of nuclease activity by the cell might allow foreign nucleic acids (viral and other) to become part of the genetic material, alter the properties of the cells, and possibly induce neoplastic transformations.

Tumor growth of Sarcoma 37 is accomplished by accelerated cellular multiplication and increased DNA (desoxyribonucleic acid) synthesis (Havas, et al, 77).

Fatty acids (octanoate, decanoate) inhibit the respiratory

activity of mitochondria in tumor slices of mouse Sarcoma 37 (Scholefield, 78). The long chain fatty acids are the more effective inhibitors, and the effects appear to be due to the fatty acids themselves. Glucose inhibits respiratory activity also.

B. Effects of Bacterial Toxins on Sarcoma 37

For more than 200 years, the observation has been made that human malignant growths may at times regress following an acute infection, usually streptococcal in nature. The active principle in these mixtures was not then known. Now many experiments have been done using the "toxins" from different strains of microorganisms as Streptococcus pyogenes alone and in combination with other strains, e.g., Serretia marcescens. These combinations have been found to be more effective. Donnelly, et al, (79) studied the effects of these mixed bacterial toxins (Serratia marcescens and Streptococcus pyogenes) given i.p. to Swiss mice bearing the tumor subcutaneously. The toxins acting on Sarcoma 37 produced edema of cells, pyknosis of nuclei, shrinkage of cells, cellular disintegration, hemorrhage, and eventual sloughing of the tumor, followed by healing and scar formation in those surviving.

It was found by other authors (Havas, et al, 77) that these same bacterial toxins are less toxic when administered to normal mice than when given to mice bearing Sarcoma 37, and that surgical removal of six day old tumors from these mice returned them from a low to a relatively normal level of resistance to these bacterial toxins.

Havas and co-workers believed that the increased susceptibility of tumor-bearing animals to the toxins resulted from toxic products originating from the treated tumors undergoing necrosis.

Tissue studies on mice given subcutaneous implants of incubated mixtures of Sarcoma 37 and Streptococcus pyogenes indicate that within 24 hours the tumor cells are surrounded by a more intense inflammatory reaction than occurs in ordinary tumor implants (Havas, et al, 80). The majority of sarcoma cells are necrotizing, but many are still viable. The inflammatory reaction increases up to one week, and there is abscess formation and necrosis. The lesions are composed of tumor cells (greatly reduced in number), cellular debris, and inflammatory exudate. By the third week, there is fibrosis and slow resolution of necrotic and inflammatory lesions. The mechanism of action by which the toxins produce tumor necrosis has not been decided. The evidence for a systemic effect on host-tumor relationships is strong. If there exists any host resistance to tumor development and growth, it may be mediated through a non-specific natural resistance or an antigen-antibody phenomenom, or both. In any case, the bacterial products that have been used for their tumor-necrotizing qualities could stimulate both of these defense mechanisms (80).

C. Effects of Viruses on Sarcoma 37

Wagner (81) treated Sarcoma 37 solid tumor fragments with (a) allantoic fluid (control), (b) WS influenza virus (non-neurotrophic), and (c) NWS virus (influenza A virus - encephalogenic varient), and

then transplanted them into mice subcutaneously. The WS strain showed no poliferation potential in this tumor and others and failed to influence their growth. The NWS virus in the transplants gradually increased in titer to the fourth day, stayed constant through day seven. At this time, the tumor weight was greatly decreased (average weight - 0.112 grams) compared to that in the control (average weight - 0.598 grams). By day ten, titer was no longer demonstrable. Between the seventh and fourteenth days, the transplants grew at an accelerated rate and circulating antibody appeared. However, the virus was lost on the tenth passage.

D. Effects of Polysaccharide Material on Sarcoma 37

Polysaccharide material from yeasts, yeast-like fungi, many bacterial microorganisms (E. coli, Candida guillermondi, etc.), and polysaccharides from higher plants and synthetic polysaccharides have been tried with varying degrees of success upon solid tumors, ascites tumors, and cells in tissue culture. Sarcoma 37 has been used in a great many of these experiments. One thing is certain, with the same polysaccharide material, the results are often quite different depending upon the kind of tumor studied; solid tumor, ascites, or tumor cells in tissue culture. The mechanism of tumor damage has been considered by some as direct and by others as indirect. Many believe, however, that the cells are directly affected as shown by their rapid swelling and edema and nuclear damage.

Creech and Hankwitz, Jr. (82) injected polysaccharide lipid complexes of S. marcescens and E. coli i.p. into eight week old Swiss mice with well established subcutaneous Sarcoma 37 (solid). They succeeded in producing major destruction of Sarcoma 37 and a high percentage of complete regression, but only after using such high levels of polysaccharide that 30 percent of their mice died from the effects. However, 50 percent of the mice remaining showed complete regression. They found that multiple injections were only rarely more effective than a single injection of polysaccharide. Diller, et al, (83), working with S. marcescens, found nuclear damage in solid Sarcoma 37 visible microscopically 1½-2 hours after i.p. injection.

Living and killed Candida species caused sloughing of Sarcoma 37 tumors (solid) in varying degrees (Maknowski, et al, 84). Candida guillermondi was found to be best with 80 percent sloughing when injected i.v.. Following i.v. injection there was widespread distribution of the organisms in normal tissues as well as in tumors, but there as no evidence of destruction of cells other than in the tumor. Also, no toxic symptoms developed in mice injected with the non-pathogenic species in doses which caused necrosis in the tumor.

Experiments were done using polysaccharides from higher plants as bryony root, burdock root, yucca leaf pulp, red clover, and others (Belkin, et al, 85). Twenty-two of 28 of these polysaccharides induced hemorrhagic necrosis in solid Sarcoma 37, transplanted intra-

muscularly in CAF albino mice. Seventeen of these polysaccharides, when injected i.p. into mice carrying Sarcoma 37 ascites tumor, produced a progressive increase in cell volume (up to 90 times that of controls) and cytoplasmic vacuolization (which became most pronounced at 72-96 hours). Another feature in Belkin's experiments was intraperitoneal leukocytosis, consisting mostly of granulocytes in different amounts depending on the polysaccharide used. By five or six days, normal cells began to appear again. Interestingly enough, some of the polysaccharides which produced hemorrhagic necrosis in solid tumors completely failed to produce swelling and vacuolization in ascites forms. Tissue cultures of Sarcoma 37 with a number of these polysaccharides yielded no results whatsoever.

O'Malley, et al, (86) reported that growth of Sarcoma 37 can be interrupted repeatedly by successive injections of S. marcescens polysaccharide. The tolerance induced by the first dose may be overcome by increasing subsequent doses to higher levels. The death rate however is high.

Most interesting results were obtained with the yeast polysaccharides, zymosan, and especially hydroglucan, on solid mouse Sarcoma 37 (Diller, et al, 83). Intravenous injection of hydroglucan was most significant. Regression of 90-95 percent of solid Sarcoma 37 was accomplished with no lethality. Administration of the same amount of hydroglucan i.p., intramuscularly, and subcutaneously produced lower percentages of regression. As in Belkin's experiments (85), the

ascites form of the tumors were not affected. Some differences were noted between bacterial polysaccharides and yeast polysaccharides. With zymosan and hydroglucan at tumor necrotizing levels, there was tumor necrosis and resorption without hemorrhage, and there were no animal deaths. In contrast, bacterial polysaccharides produced vascular damage, hemorrhage, and numerous deaths. It is felt by Diller's group (83) that the mechanism of action of yeast polysaccharides may not be the same as that of bacterial polysaccharides. The most notable feature of tumor regression with hydroglucan is the increased production of phagocytic cells not observed in some mice whose tumors are not regressing. Apparently, there is a host-mediated reaction here which involves stimulation of the phagocytic elements of liver and spleen, and it is a matter of host response or failure to respond to yeast polysaccharides which determines tumor regression. Weiss, et al, (83) discussed evidence for the assumption that the ability of malignant growths to progress in the host reveals failure of antibody formation of phagocytic properties of the RE system.

E. Drugs and Chemicals on Sarcoma 37

Different chemical classes of drugs will damage tumor cells, and there is no one group outstandingly effective. For instance, aminopteropterin in doses of 0.5 or 1 mg/kg and thio-TEPA in doses of 1-2 mg/kg i.p. showed their toxic activity by consistently decreasing free tumor cell concentration and the amount of exudation in ascites

Sarcoma 37 (87). Way, et al, (88) showed that 8-azaguanine inhibited Sarcoma 37 in CAF mice in large doses.

Table III lists experimental results with different chemicals (Shear, et al, 89,90; Beck, et al, 91).

F. Hormones on Sarcoma 37

Hormones may act beneficially or detrimentally on Sarcoma 37.

1. Sex Hormones: Studies were done on the growth of ascites tumor cells in male mice previously treated with sex hormones (Goldie, et al, 92). It was determined that extended preparation with high doses of androgens alone or androgens alternated with estrogens induced in male mice a hormone imbalance which conditioned, for a limited time, the peritoneal cavity for increased spread and growth of ascites tumors. The concentration of free tumor cells was found to be less in estrogen-treated animals and in controls.

2. Adrenal Cortical Hormones: In the work of Diller and others (93) in Sarcoma 37 transplanted subcutaneously in Carworth Farm mice, it was demonstrated that i.p. injection of adrenal cortical extract was capable of producing degenerative changes in the tumor and sloughing, especially in the female. There was a decrease in the size of the tumor following injection of adrenal cortical extract in males and females, but this inhibition of growth could not be maintained in the males, even with repeated doses of the extract. However, there was some spontaneous regression and sloughing showing that Sarcoma 37 was somewhat precariously established in this strain. Further work of

Diller and associates (94) showed the following interesting results:

1. In Sarcoma 37 tumor bearing mice (subcutaneously), adrenal cortical extract injected i.p. produced degenerative changes in the adrenal gland, especially in the medulla as well as in the subcutaneous tumor. There appeared to be greater damage in the adrenals of females injected with cortical extract than in males.
2. Intra-peritoneal injection of S. marcescens polysaccharide into mice, bearing Sarcoma 37 subcutaneously, produced degenerative changes in the tumor within six hours and rapid sloughing in one of every four mice. However, an equal number died from the treatment. At the same time, this polysaccharide produced hemorrhage and degenerative changes in the adrenal, again especially in the medulla.
3. Simultaneous administration of the polysaccharide and adrenal cortical extract decreased the toxic effect, i.e., more animals survived. There was also a minimal effect in the adrenal and a delayed or slower tumor breakdown, 24 hours instead of six hours. The adrenal medulla and cortex both appeared relatively normal by 24 hours.
4. Interestingly enough, in non-tumor animals, or in animals with tumors that do not respond to these agents

through tumor degeneration, the adrenal damage following the administration of the polysaccharide alone, the adrenal cortical extract alone, or both together appeared to be negligible.

TABLE II

SURVIVAL TIMES OF MICE AND ROUTES OF INJECTION (SARCOMA 37)

Modified after Goldie, et al, 68

SURVIVAL TIMES IN DAYS

	Intra-pleural	Flank	Back	Foreleg	Hindleg	I.P.
Sarcoma 37	6(4-9)	14(11-20)	19(13-25)	10(9-13)	9(7-12)	8(7-8)

TABLE III

CHEMICAL AGENTS
WITH POTENCY IN PRODUCING DAMAGE
IN SARCOMA 37

Modified from Shear, et al, 89, 90
and Beck, et al, 91

Chemical Class	No. Comp. Exam.			No. Yielding Results		
Quaternary ammonium salts	283	87	(90)	13	8	(90)
Acridines	41	41	(90)	7	4	(90)
Phenazines	30			3		
Sulfonamides	12			0		
Unsaturated ketones	34			3		
Isoquinolines	17			0		
Quinones	30			8		
Stilbenes	17			5		
Alkaloids	60			4		
Di & Tri Phenyl methanes	40			2		
Amidines and quanidines	24			1		
Arsenicals (90)	63			6		
Aromatic tri-valent Arsenicals						
(90)	25			5		
(91)	24			6		

VII. Related Experiments

Several experiments have shown that transformation of Cortisol to its products is different in normal cells than in different types of abnormal cells.

A. Normal Connective Tissue

That the fibroblast is the main site of cortisol (F) metabolism is supported by the following experiments from our laboratory (29,64, 95). Cortisol C^{14} was incubated with normal AK mouse loose connective tissue. Several compounds were isolated and identified, including mainly cortisone (E), 20 epi substance "E" of Reichstein, a slight amount of dihydrocortisol, Reichstein's "U", corticosterone, and 11BOH androstenedione. The results indicated that normal fibroblasts preferentially oxidize position C-11 of the cortisol molecule to form cortisone. The main changes were seen to be oxidation and reduction of the substituted groups on the gonane nucleus. Since the fibroblast makes up 90 percent of connective tissue, one may conclude that it must be this cell that performs these conversions.

B. Tissue Culture Cells

Many experiments showed (28,35,39,95,96), first, that uterine fibroblasts in tissue culture have a greater ability to reduce the C-20 position than do normal fibroblasts. Also, androstenedione was formed in greater amount than in normal connective tissue and cortisone was

not the main product of conversion as in normal connective tissue. It was found that these fibroblasts, when incubated with cortisol C^{14}, formed cortisone, Reichstein's "E" and "U", corticosterone, and 11BOH androstenedione; however, Reichstein's "E" was mostly in the B form.

Second, a resistance strain of uterine fibroblasts was found to metabolize twice as much cortisol per unit time as did a sensitive strain (97,98). When cortisol C^{14} was incubated with two strains of uterine fibroblasts, one of which was resistant to the growth inhibition of cortisol (U12-35) and the other sensitive to cortisol growth inhibition (U12-79), it was found that both strains metabolized cortisol to steroid products which did not differ significantly in either types or relative quantities, but that the resistant strain metabolized more cortisol per unit of time.

Third, it has been shown (99) that cortisol is the active form of the steroid molecule, whereas other products are less active. The metabolites produced from cortisol C^{14} incubation with uterine fibroblasts were shown to be less inhibitory to growth than cortisol itself. Therefore, the more cortisol converted to inactive products, the less active cortisol there was to cause destruction of fibroblasts or inhibit their growth.

C. Fibrosarcoma Cells

Products not occurring in normal fibroblast conversion may occur in fibrosarcoma cell conversion. In experiments with mice (Berliner, 100), cortisol was found to be converted by malignant cells to cortisone, sub-

stances "E" and "U" of Reichstein, corticosterone, dihydro F, and 11BOH androstenedione. Some 11 keto-etiocholanedione, which is not seen in normal connective tissue, was formed also. Fibrosarcoma cells metabolized cortisol to a greater extent than did normal fibroblasts.

D. Osteosarcoma Cells

Osteosarcoma cells of dogs were found to catabolize cortisol C^{14} and the metabolites formed were found to be the same as those produced by normal fibroblasts, but they were produced in greater amounts (39, 101). Quantitative differences were seen in metabolic products of incubated fresh osteosarcoma cells and those grown in tissue culture. Tissue culture osteosarcoma cells reduced cortisol at position 20 in a greater amount than fresh cells, but oxidized C-11 to a lesser degree. Also, the tissue culture osteosarcoma cells incubated with cortisone C^{14} were not able to reduce the molecule to cortisol, whereas the freshly isolated osteosarcoma cells could (102).

E. Malignant Lymphocytes

The malignant lymphocytes have been relatively extensively investigated (39,58,62,103,104,105,106). In mice, malignant lymphocytes transformed the active cortisol molecule to metabolic products which were incapable of producing lymphocyte destruction or regulating lymphocytopoiesis. When malignant lymphocytes were incubated with cortisol C^{14}, cortisol converted to cortisone, substances "E" and "U" of Reichstein, and 11BOH androstenedione. They had the ability to reduce the

C-20 keto-group and oxidize the side chain to form C-19 compounds in very large amounts. Normal lymphocytes performed the same conversions, but in small amounts. In mice, immature lymphocytes and lymphocytes after repeated large doses of cortisol were also found to have an increased capacity to metabolize cortisol over lymphocytes from normal mice, but they did not do so to the same extent as did malignant lymphocytes.

F. Studies in Man

When cortisol metabolism was compared in a normal subject and in a patient with acute lymphatic leukemia, it was observed that 11BOH etiocholanolone (from 11BOH androstenedione) was greatly increased in the urine of the leukemic patient over that of the normal person (100,107, 108). Years ago, Dobriner (109) studied patients with lymphatic leukemia and other cancers and had found this substance to be present in their urine. At that time, however, his methods did not pick it up in normal urine.

Slaunwhite, et al, (110) studied patients with prostatic cancer, and noted that they excreted large amounts of 11-oxygenated-17-ketosteroids upon receiving cortisone. Normal individuals receiving the same quantity of cortisone excreted only small amounts. He showed similar results in a lung cancer patient.

Sokal and Buchwald (111) gave cortisol acetate to patients with advanced carcinoma of the prostate and noted that they excreted greater amounts of 17-ketosteroids than did normal persons treated similarly.

In other carcinomatous conditions, 17-ketosteroids were found in large amounts in the urine, while they were rare in normal persons.

Other investigators (112) reported that patients with estrogen resistant tumors catabolized estradiol $_4C^{14}$ to a greater extent than did patients with estrogen sensitive tumors.

THE EXPERIMENT

The present experiment investigates the interconversion of labeled cortisol and cortisone in both normal connective tissue of mice and in Sarcoma 37 of hamsters.

Many experiments have been done with normal connective tissue cells and abnormal cells using cortisol (F_4C^{14}) or using cortisone (E_4C^{14}), noting the ability of the cells to metabolize either of these steroids. The present experiment labels not only the cortisol (F) molecule and follows its products of conversion, but at the same time labels cortisone (E) and follows its metabolic pattern. Equimolecular quantities of cortisol-7-H^3 (F-7-H^3) and cortisone-4-C^{14} (E_4C^{14}) were added to the incubation mixtures of fibroblasts of mice and Sarcoma 37 cells of hamsters, providing information on the conversion of cortisol to cortisone, cortisone to cortisol, and conversion products from cortisol, conversion products from cortisone, etc.

I. Materials and Methods

A. Subjects and Tissues

1. Normal Connective Tissue: One hundred twenty-eight male and female AK mice bred in our laboratory were weaned at 8-10 weeks of age. They were mixed and divided into two groups of 64 animals each. One group was sacrificed one day, the second group the next. Each group was sacrificed by neck fracture, then a dorsal air pocket was quickly

made and the underlying connective tissue was removed. The connective tissue from eight animals was used for each incubation, together with equal amounts of a hormone mixture containing 1μm of cortisone-4-C^{14} (E_4C^{14}) and 1μm of cortisol-7-H^3 (F-7-H^3), and 10 cc of phosphate buffer at pH 7.4.

2. Sarcoma 37: Ten golden hamsters with Sarcoma 37, from a colony with Sarcoma 37 from the University of Rochester Medical Center, Rochester, New York, were used in this part of the experiment. The hamsters, five males and five females, were inoculated with Sarcoma 37 in the subcutaneous dorsal area when they were 44 days old. They were received the next day. The hamsters had been fed on Purina Lab Chow with lettuce every 5-6 days. In our laboratory, they were fed a mixture of Purina Lab Chow pellets and Friskies Dog Cubes, and a mixture of wheat, liver oil, and meal; also lettuce every few days. Within a week, a slight growth of the tumors could be detected. By two weeks, the tumor was at least 3 cm. in diameter. Fifteen days after tumor implant, six hamsters were sacrificed by decapitation. Two tumors were placed in 10 percent formalin to be studied histologically. See figure 3. The tumors were put together, weighed, minced, and incubated. Each incubation contained 300 mg. of tumor tissue with equal amounts of a hormone mixture of 1μm of cortisone-4-C^{14} and 1μm of cortisol-7-H^3 and 10 cc of phosphate buffer at pH 7.4.

B. Methods of Extraction

The incubations were stopped at various time intervals; 1, 5, 15,

30 minutes, and 1, 2, 3, 4 hours in the case of normal tissue; and only through 3 hours with Sarcoma 37, since it was found that by four hours, most of the cells had died or been destroyed. Zero and 3 or 4 hour controls of the hormone mixture, plus buffer, without tissue were also carried through to detect changes during the incubation procedure. The incubations were done at 37° C temperature with constant movement, and a duplicate of each incubation flask was always made. When the incubation was completed, acetone was added to stop the metabolic processes.

The contents of each flask were homogenated and extracted three times with hot acetone, passed through a filter paper, and evaporated in a vacuum. The aqueous residue was re-extracted with chloroform three times and evaporated to dryness. This dried residue was transferred into a centrifuge tube, using a 1:1 mixture of chloroform-methanol. The mixture was dried under nitrogen. To each extract 100 each of non-radioactive cortisol and cortisone were added as carriers in order to detect the positions of the hormones on paper under ultraviolet light. One dimensional descending paper chromatography was done, using the Zaffaroni methods. Strips 2.5 cm. were used in normal connective tissue, and strips 3.0 cm. in sarcoma tissue. The solvents used were hexane, overnight, to eliminate the fat, and chloroform, to drop from the strip for one hour. Isolations and identifications were made following the techniques described by Berliner (113,114). Equal paper areas corresponding to the sites of absorption of cortisol and cortisone were cut out and eluted with methanol directly into scintil-

lator vials and dried under nitrogen. Other areas corresponding to Reichstein's "E" and "U", C-19 metabolites, and the droppings were eluted with methanol into scintillator vials and dried under nitrogen also. Twenty ml. of scintillator fluid was freshly prepared (1) and added to each vial, which was then counted in a scintillator. Since each vial may contain both tritium and carbon 14, the following procedure was used to evaluate the radioactivity in each vial.

(1) Scintillator fluid - P.P.O. - 4 gms/liter + POPOP - 100 gms/liter of toluene

C. Analysis of Data

The counting of the samples containing carbon 14 and tritium was done in a Packard Tri-Carb Liquid Scintillation Spectrometer, Series 314A. The following settings were used: High voltage tap 6.00 (1150 volts), analysis mode 2 split. Disciminator settings are AA^1=10 volts, B=50 volts, and C=70 volts. With these settings, H^3 is counted in channel I (10-50 volts) and C^{14} in channel II (70-volts). If it were not for the fact that some tritium counts spill over into channel II, the calculation would be much simpler.

Since we have carbon 14 in channel I and tritium in channel II, we must go to simultaneous equations for calculating the amount of carbon 14 and tritium in each channel. In order to do this, a standard of pure C^{14} and a standard of pure H^3 are required -

Let A = count rate Tritium Standard

Leb B = count rate Carbon^{14} Standard

Let K = count rate of the sample

X = the fraction of standard A in the specimen

Y = the fraction of standard B in the specimen

In the specimen, the sum of the individual count rates = total count rate.

$A_1X+B_1Y = K_1$ in channel I

$A_2X+B_2Y = K_2$ in channel II

Therefore:

$$X = \frac{K_1B_2 - K_2B_1}{A_1B_2 - A_2B_1} \qquad (1)$$

$$Y = \frac{K_1A_2 - K_2A_1}{B_1A_2 - B_2A_1} \qquad (2)$$

This form of the equation lends itself very readily to solution by high speed digital computers.

For those who do not have access to a computer, the following forms are more adaptable for solution, using a desk calculator.

$$X = \frac{K_1 - \frac{B_1}{B_2} K_2}{A_1 - \frac{B_1}{B_2} A_2} \qquad (3)$$

$$Y = \frac{K_2 - \frac{A_2}{A_1} K_1}{B_2 - \frac{A_2}{A_1} B_1} \qquad (4)$$

In the experiments described below, a Burroughs 205 Datatron Computer was programmed in such manner that all calculations involved in solving the above equations (1 and 2) were made in one operation.

X = the percentage of the H^3 standard in the sample based on radioactivity, and Y = the percentage of the C^{14} standard in the same sample based on radioactivity. The computed results may be seen in Tables IV, V, VI, and VII for the time intervals studied.

Compounds analyzed include cortisol (F), cortisone (E), and Reichstein's "E" and "U", and C-19 metabolites.

The results were then plotted on bar graphs in percent. See figures 5 - 10.

II. Results

Histological examination of tissue slices and pictures confirmed that the tumor was a sarcoma. See figure 3.

The results of the experiment are summarized in figure 4 and figures 5 - 10. The results of the experiment are summarized in Tables IV through VII.

A. Conversion of Cortisol (F)

1. Conversion of Cortisol (FH_3) to Cortisone (EH_3): In normal connective tissue, cortisol is converted to cortisone. The amount of conversion increases with time to about 20 percent at two and three hours. In sarcoma tissue, cortisol is also converted to cortisone, but

in very small amounts. The conversion reaches its height at two hours when it is approximately three percent. It drops off at three hours to about one percent. These data are represented in figure 5.

2. Conversion of Cortisol (FH_3) to Other Products: In normal connective tissue, cortisol is converted to other products also; Reichstein's "U", Reichstein's "E", and C-19 metabolites. Conversion to these products considered together depends on time. At 15 minutes, it reaches its peak at about six percent and decreases at 30 minutes to four and one-half percent. It remains at about five percent during the remainder of the experiment. All in all, conversion does not change greatly. Sarcoma 37 conversion of cortisol to all products but cortisone increases suddenly at five minutes to 12 percent, from about three percent at one minute. This drops at 15 minutes to about three percent and increases slowly to about six percent at two hours. However, there is a large increase by three hours to 21 percent. These data are represented in figure 6.

3. Conversion of Cortisol (FH_3) to C-19 Metabolites Alone: In normal connective tissue, the conversion of cortisol to C-19 metabolites plus droppings is small. It ranges from 1½ to nearly three percent, averaging two percent at three hours. Conversion of cortisol to C-19 metabolites in sarcoma is also small. The greatest increase in conversion above that of normal tissue is at five minutes, when it increases to about nine percent. It then drops to about 1½ percent at 15 minutes, slowly rises, and levels off between three and four percent at two and

three hours. These data are represented in figure 7.

B. Conversion of Cortisone (E)

1. Conversion of Cortisone (EC^{14}) to Cortisol (FC^{14}): In normal connective tissue, conversion of cortisone to cortisol increases with time up to 40 percent at three hours; the greatest increase, from 10 percent to 40 percent, occurring during the last hour. In sarcoma, the amount of conversion of cortisone to cortisol is very small, reaching only a little over four percent at three hours. This conversion appears to be the one of importance in distinguishing sarcoma cells from normal cells. These data are represented in figure 8.

2. Conversion of Cortisone (EC^{14}) to Other Products: In normal connective tissue, there is conversion of cortisone to other products, i.e., Reichstein's "E" and "U", and C-19 metabolites. Considered together, these products are formed in greater amount than the identical products from cortisol. (Compare figures 6 and 9.) In normal tissue, Reichstein's "U" and "E", and C-19 metabolites are already formed at one minute, and in general, they slowly increase to about 12 percent by three hours. In sarcoma, the picture is radically different. At one minute, the conversion products are similar in amount to the normal tissue, but by five minutes, the products in sarcoma suddenly increase to 40 percent, compared to nine percent in normal tissue. Then the products drop to about 25 percent at 15 minutes and remain relatively level for the rest of the three hour incubation. These data are represented in figure 9.

3. Conversion of Cortisone (EC^{14}) to C-19 Metabolites Alone: In normal connective tissue, C-19 metabolites, plus droppings, are not formed in large amounts. They average about six percent from one minute to three hours. However, the amount of C-19 metabolites formed is greater than that formed from cortisol, which averages less than three percent. (Compare figures 7 and 10.) In sarcoma, the picture is again very different. At one minute, the amount of C-19 metabolites formed is about one percent. By five minutes it increases to 38 percent. At 15 minutes it decreases to about 24 percent and stays there through one hour. This decreases at two hours to about 10 percent, but increases again to 20 percent at three hours. These data are represented in figure 10.

III. Discussion

This experiment confirms that fibroblasts in loose connective tissue are capable of converting cortisol to cortisone and cortisone back to cortisol, and that loose connective tissue fibroblasts are capable of reducing the C-20 keto position to H-C-OH and oxidizing the side chain to form C-19 metabolites. In normal fibroblasts, conversion to C-19 metabolites occurs in small amounts, while in sarcoma cells it occurs in large amounts. Also, there is greater inactivation of the active molecule cortisol in sarcoma cells. Further, the experiment shows: (1) That conversion products from cortisone are formed in greater amounts than those from cortisol in both normal and sarcoma cells; (2) these conversion products occur in much greater amounts in

sarcoma cells than in normal cells; and (3) in normal connective tissue, conversion products are formed almost immediately, whereas in sarcoma cells, the formation of conversion products always lags in conversions from cortisol to cortisone and cortisone to cortisol.

First, consider the conversion from cortisol to cortisone. In normal mouse fibroblasts, the rate of conversion of cortisol to cortisone (C-11 oxidation) is detectable in the first five minutes of incubation and is fairly large in amount. However, in sarcoma the rate of conversion is not detectable until two hours of incubation, and even at this time the amount formed is very small (figure 5).

The conversion of cortisone to cortisol (C-11 reduction) is represented in figure 8. Conversion is first seen in normal cells by 15 minutes, whereas conversion by sarcoma cells does not appear until one hour. The rate of reduction by sarcoma cells is again extremely small as compared with that of normal cells. This C-11 reduction appears to be the important conversion in sarcoma cells since it shows that very little cortisone is converted to the active form cortisol. Therefore, little cortisol is available to the sarcoma cells. It is obvious that the rate of conversion of cortisol to cortisone and cortisone to cortisol, or the ratio of interconversion in sarcoma cells is much different from that in normal cells.

Conversion of cortisol H^3 to other products, except cortisone, in normal tissue appears at one minute and increases slightly over a three hour period. (See figure 6.) In sarcoma tissue, conversion products

also appear by one minute, but rise suddenly above those of normal tissue at five minutes, and decrease to almost the level of normal tissue until two hours. Between two and three hours, there is a large increase to four times that found in normal tissue.

In normal tissue, conversion of cortisone C^{14} to other products, except cortisol, appears at one minute and rises slowly. (see figure 9.) Conversion products from cortisone are formed in a greater amount than those from cortisol. The amount of conversion products from cortisone C^{14} in sarcoma cells is greatly different. Even at one minute conversion products are formed in greater amount than in normal cells. By five minutes the amount of conversion products rises to over four times that found in normal cells. Conversion products continue to be formed in great amount, leveling off at three hours to about twice that of normal cells. Over the three hour period, conversion products from cortisone C^{14} in sarcoma cells are again formed in greater amount than from cortisol H^3 in sarcoma cells over the same period. (See figures 6 and 9.)

The picture is similar when we consider the formation of C-19 metabolites from cortisol H^3 and cortisone C^{14}. In normal tissue, the conversion of cortisol H^3 to C-19 metabolites is small, appearing at one minute and remaining about the same over the three hour period. In conversion from cortisone C^{14}, the formation of C-19 metabolites is somewhat increased, but not greatly. It appears at one minute and varies only slightly over the three hour period.

In sarcoma tissue, conversion from cortisol H^3 appears at one minute and is very small and below that of normal tissue. It rises at five minutes to over three times that of normal tissue, drops down at 15 minutes and again rises above normal connective tissue at 30 minutes, and continues so to three hours.

Conversion from cortisone C^{14} in sarcoma tissue appears at one minute in small amount and below that of normal tissue, rises at five minutes to far above that of normal tissue. It drops at 15 minutes but still remains far above normal tissue, and continues in much greater amount than that found in normal tissue to three hours. Again the total conversion over the three hour period from cortisone C^{14} to C-19 metabolites is much greater than the total conversion from cortisol H^3 to C-19 metabolites.

Enzyme systems are essential to the transformations of cortisol by fibroblasts and other cells. These include 11BOH dehydrogenase systems and the presence of co-factors as DPN, TPN, DPNH, and TPNH (30). Therefore, the availability of a particular co-factor and whether it is oxidized or reduced form will determine the amount of metabolism to active or inactive steroid hormones. Talalay and his workers (115) considered that the steroids act as hydrogen donors and acceptors, participating in the reversible oxidation and reduction of diphospho and triphospho pyridine nucleotide.

Previous studies and the results of this experiment show that malignant cells do metabolize steroids to inactive metabolic products

to a greater extent than do their normal counterparts, and that the presence of tumor or leukemia favors the splitting of the side chain with the formation of C-19 compounds.

This experiment shows that it is C-11 reduction that is particularly or especially impaired in sarcoma cells.

Other experiments have labeled either cortisol or cortisone and traced the metabolic products from one or the other. The present experiment labels both cortisol and cortisone in mouse and hamster tissue and shows not only the metabolic products from cortisol and from cortisone, but also the rate and amount of interconversion of these two hormones in both malignant and normal cells. Moreover, labeling of both cortisol and cortisone in normal and malignant cells clearly depicts that the major defect in sarcoma cell conversion is in C-11 reduction.

This C-11 reduction appears to be the conversion of great importance in distinguishing the metabolism of sarcoma cells from that of normal cells. These sarcoma cells have somehow lost the capacity to convert a large amount of the potentially active form cortisone to the active form cortisol, and therefore the amount of cortisol available is far less than that required to produce the same effect as in normal cells. Thus, control over cell growth and other processes may be lost.

Knowing about rates and amounts of interconversion in normal and malignant tissues is basic for devising methods of interfering with abnormal metabolism in malignant cells.

It has been found that substitution of a methyl group on the second carbon of cortisol and of cortisone prevents their interconversion in vitro and in vivo by such cells as fibroblasts and lymphocytes (111). Thus, 2-methyl cortisol is even more anti-inflammatory than cortisol itself, and it could very likely be more effective than cortisol on malignant cells. In fact, other substitutions on the steroid nucleus, as in fluocinolone acetonide, may be even more effective on malignant cells than naturally occurring hormones.

Possible explanations for the difference between normal and sarcoma cells observed here might be as follows: (1) Enzymatic defects or co-factor imbalance or both could affect biotransformation and thus the ability of cells to inactivate hormones; (2) changes in cell membrane permeability might selectively inhibit or increase contact of steroids with the necessary enzyme-co-enzyme system (39); (3) changes in nucleic acids may occur, thereby altering the genes themselves; (4) inhibitory factors may no longer enter the cells (116); (5) hormone binding with proteins or other substances may make the steroids unavailable at the cell surface (117,118); and (6) minimal C-11 reduction in sarcoma cells, as shown in this experiment, may enable these tumor cells to inactivate cortisol faster than in normal cells, enable them to convert more easily that which is present in inactive forms, influence their resistance to increased amounts of hormone, and influence the requirement of greater quantities of cortisol than normal cells to produce the same effect. On the other hand, normal cells

through active C-11 reduction can maintain more molecules in the active state and so keep sufficient cortisol available to protect the animal against stressors and maintain the homeostasis so necessary to the normal functioning of the organism.

Several things appear to me to be very clear:

1. Since conversions of cortisol to cortisone in sarcoma tissue are delayed (they do not appear for two hours) and are very small in amount (one to three percent); and

2. Since conversions of cortisol to cortisone in normal connective tissue appear very rapidly (within five minutes) and are large in amount (20 percent); and

3. Since conversions of cortisone to cortisol in sarcoma tissue are again delayed (they do not appear for one hour) and are again very small in amount (four percent); and

4. Since conversions of cortisone to cortisol in normal connective tissue appear quite rapidly (15 minutes) and are large in amount (40 percent); and

5. Since conversion products from both cortisol and cortisone appear immediately (within one minute) in both normal and sarcoma tissue; and

6. Since the 11BOH dehydrogenase systems and the co-enzyme DPN-DPNH or TPN-TPNH are considered necessary for the conversions of cortisol to cortisone and cortisone to cortisol, then

It seems obvious that the co-enzymes may have been destroyed,

greatly incapacitated or inactivated, missing, altered, or in some way prevented from contact with the steroids cortisol and cortisone. Consequently, the normal pathway of cortisol to cortisone or cortisone to cortisol in sarcoma cells seems to have been circumvented, and a new or altered pathway may have permitted inactive metabolic products to be formed rapidly and in large amounts.

Therefore, it is suggested that sarcoma experiments in which DPNH+ or TPNH+ is injected with the tumor cells and after the tumor has begun to grow might prove most interesting and possibly enlightening. Co-enzymes DPNH+ or TPNH+ are suggested because in C-11 reduction more of the active steroid may be made available to prevent or decrease the rapid metabolic inactivation of this steroid which may lead to or may be a consequence of cell malignancy.

Further, work should be continued on the nature and amounts of the C-19 metabolites formed in both the normal and especially in sarcoma cells

Finally, the success of Diller's work with hydroglucan on the sloughing of 90-95 percent of solid S-37 tumors in mice without lethality suggests that experiments should be done using hydroglucan or possibly glucan alone and using one or the other together with DPNH+ or TPNH+. Also study of glucan, with the aim of determining its mechanism of action, would prove most interesting as would further investigations on the possible antigen-antibody relationships which may be involved in tumor production and destruction as discussed here.

IV. Summary

The present experiment was designed to investigate a part of the connective tissue steroid metabolism, which is so important to the normal physiological state of an organism.

Several conclusions can be drawn from this experiment:

1. The rate of interconversion from cortisol to cortisone and from cortisone to cortisol is very different in sarcoma cells than in normal cells.

2. In conversions of cortisol to cortisone and cortisone to cortisol, the rate of biotransformation begins more slowly in the malignant cells. Perhaps for some reason cortisol and cortisone are unable to enter malignant cells as rapidly as normal cells, or the enzymes necessary to the reaction have been incapacitated or inactivated.

3. The conversion to inactive products is much greater in sarcoma cells than in normal cells, so that sarcoma cells seem to squander the biological material necessary to protect the organism against stressors.

4. In the present experiment, conversion to inactive products is greater from cortisone C^{14} than from cortisol H^3, especially in sarcoma cells.

5. In normal cells, C-11 oxidation is prominent (20 percent at three hours), and it is second only to C-11 reduction which is twice as great (40 percent at three hours).

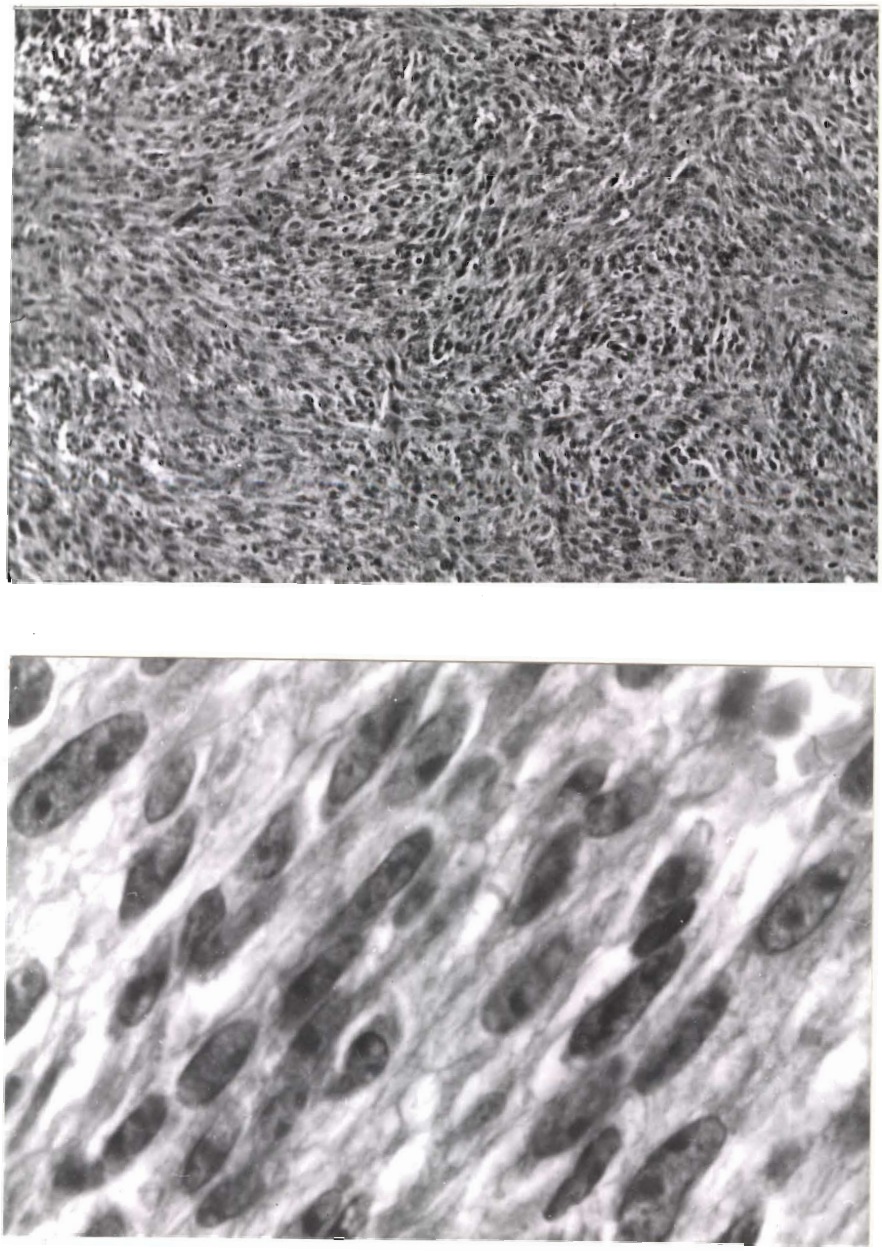
6. In sarcoma cells, C-11 oxidation is very small (1-3 percent

at three hours), so also is C-11 reduction (4 percent at three hours).

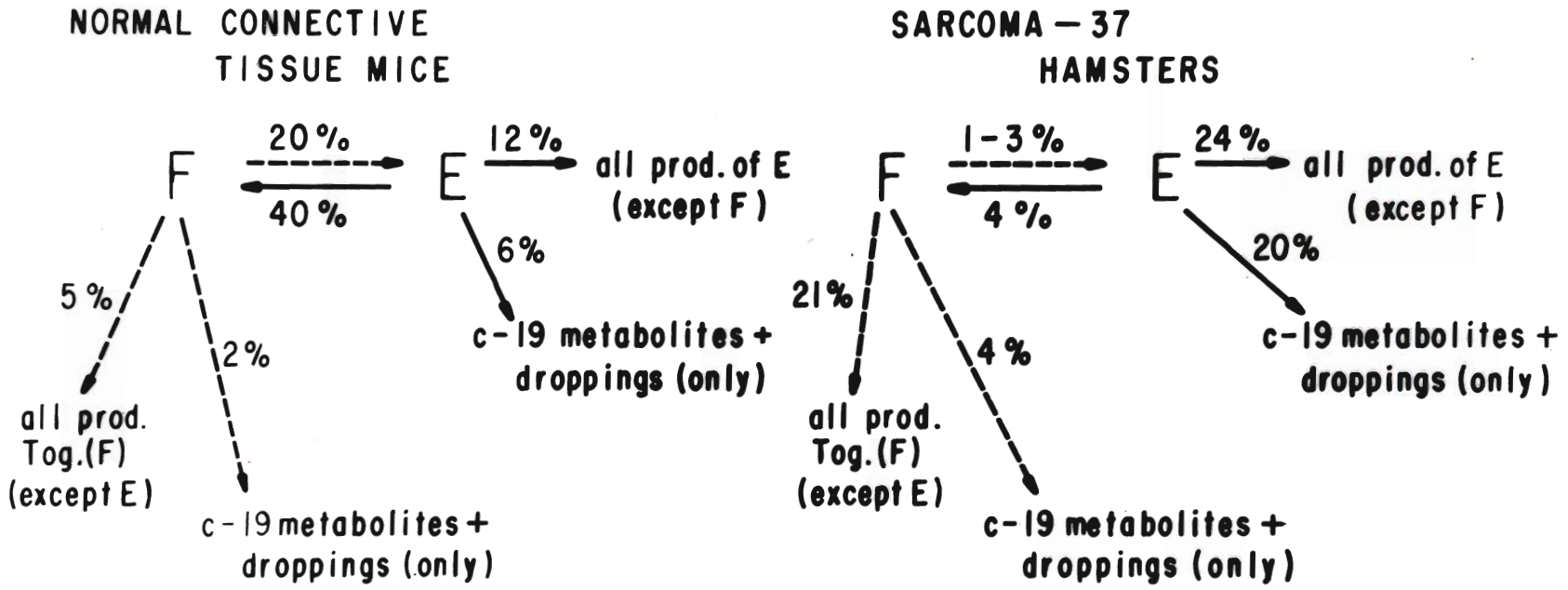
7. In normal cells, C-11 reduction is tremendous. This enables normal cells to keep sufficient cortisol molecules in the active state to protect the animal against stressors and maintain the homeostasis necessary to the normal functioning of the organism.

8. However, sarcoma cells have lost the capacity to convert a large amount of the potentially active cortisone to cortisol (C-11 reduction). Thus, the amount of cortisol available is less than in normal cells and control over growth and other processes may be lost. If this applies to the whole animal, peripheral fibroblastic-induced adrenal cortical insufficiency would be produced, leaving less cortisol present to protect the organism against inflammatory disease and noxious or chemical substances. In this way, the homeostasis so necessary to the normal state is disrupted.

If we can discover at what point in normal fibroblast metabolism the capacity of C-11 reduction is lost, we may come closer to the final enigma of the cancer process and thereby have a better chance to control it. But to do this, it is necessary to understand the cell itself and its environment and to understand the metabolism of normal and of malignant cells. By furthering these studies we will be better equipped to develop synthetic hormones and other means which may cause destruction of carcinomatous lesions without being toxic to the organism.



(3) Histological Appearance of Sarcoma 37



SUMMARY OF EXPERIMENT RESULTS AT 3 hrs.

(4)

TABLE IV

SUMMARY OF EXPERIMENTAL RESULTS

NORMAL CONNECTIVE TISSUE

	0 Control Average	4 hrs. Control Average	1 min. Average	5 min. Average	15 min. Average	30 min. Average	1 hr. Average	2 hrs. Average	3 hrs. Average
Area I - R. "E"	*0.02352	0.06630	0.01628	0.02335	0.01706	0.01779	0.02452	0.01890	0.02114
	**0.01688	0.02686	0.00804	0.01363	0.01486	0.01434	0.01722	0.01308	0.01980
Area II - FH^3	*Std.	0	0	0	0.02682	0.04435	0.09234	0.10294	0.40064
	**Std.	0.91947	1.06160	1.06293	0.97779	0.96120	0.87113	0.81114	0.80402
Area III - R "U"	*0.00900	0.00600	0.00600	0.00800	0.01216	0.01072	0.02046	0.02829	0.04618
	** 0	0	0.00500	0.00500	0.02533	0.00909	0.00915	0.01011	0.01406
Area IV - EC^{14}	*Std.	0.88911	1.08898	1.03549	1.04976	1.05717	1.05524	0.86043	0.61483
	**Std.	0	0	0.01520	0.04456	0.06922	0.18523	0.21612	0.19758
Area V - C-19 Metabolites	*0.08358	0.04139	0.03862	0.03428	0.04122	0.03099	0.01518	0.06204	0.03974
	**0.02206	0.01194	0.00525	0.00917	0.01298	0.00761	0.00764	0.02096	0.01128
Area VI - Droppings	*0.01214	0.04935	0.02660	0.02326	0.02382	0.02876	0.05254	0.01164	0.02132
	**0.00174	0.02227	0.01177	0.01305	0.01227	0.01528	0.02032	0.00464	0.00805
Areas V & VI - Total	*0.09572	0.09074	0.06522	0.05754	0.06504	0.05975	0.06772	0.07368	0.06106
	**0.03800	0.03421	0.01702	0.02222	0.02525	0.02289	0.02796	0.02560	0.01933

* - EC^{14} Cortisone
** - FH^3 Cortisol

TABLE V

SUMMARY OF EXPERIMENTAL RESULTS
SARCOMA 37

		3 hrs. Control Average	1 min. Average	5 min. Average	15 min. Average	30 min. Average	1 hr. Average	2 hrs. Average	3 hrs. Average
Area I - R. "E"	*		0.00364	0.02350	0.01825	0.02358	0.01639	0.01704	0.00790
	**		0.00302	0.01950	0.00869	0.01239	0.01010	0.01844	0.00570
Area II - FH^3	*	0.01178	0	0	0	0	0.01884	0.03789	0.04427
	**	1.00497	0.98992	0.80904	0.98308	0.97048	0.96995	0.86424	0.71967
Area III - R. "U"	*		0.06340	0.00510	0.01172	0.00819	0.01972	0.14316	0.02740
	**		0.00786	0.00864	0.00737	0.00650	0.00372	0.00802	0.17080
Area IV - EC^{14}	*	1.07056	1.12715	0.71152	0.93928	0.97820	0.93306	0.91867	0.92667
	**	0	0	0	0	0	0	0.02948	0.01080
Area V - C-19 Metabolites	*		0.02894	0.25518	0.16666	0.14124	0.15037	0.05440	0.01576
	**		0.00732	0.00915	0.00638	0.01288	0.01298	0.02354	0.02488
Area VI - Droppings	*		0.01250	0.12220	0.07011	0.09005	0.08546	0.05296	0.04492
	**		0.00339	0.08169	0.01078	0.01738	0.01516	0.01493	0.01144
Total	*		0.04144	0.37738	0.23677	0.23129	0.23583	0.10736	0.20252
	**		0.01071	0.09084	0.01716	0.03026	0.02814	0.03847	0.03632

* - EC^{14} Cortisone
** - FH^3 Cortisol

TABLE VI

SUMMARY OF EXPERIMENTAL RESULTS
H^3 PRODUCTS

NORMAL TISSUE

Time Min.	R. "E"	R. "U"	C-19 Met. + Dr.	All Except E	F to E
1 min.	0.8	0.5	1.7	3.0	0
5 min.	1.3	0.5	2.2	4.0	1.5
15 min.	1.5	2.5	2.5	6.5	4.4
30 min.	1.4	0.9	2.3	4.6	6.9
1 hr.	1.7	0.9	2.8	5.4	18.5
2 hrs.	1.3	1.0	2.5	4.8	21.6
3 hrs.	1.9	1.4	1.9(2%)	5.2(5%)	19.7(20%)

SARCOMA TISSUE

1 min.	0.3	0.78	1.0	2.0	0
5 min.	1.9	0.86	9.1	11.8	0
15 min.	0.87	0.73	1.72	3.34	0
30 min.	1.2	0.65	3.0	4.85	0
1 hr.	1.0	0.37	2.8	4.2	0
2 hrs.	1.8	0.8	3.8	6.4	2.9)
3 hrs.	0.57	17.1	3.6(4%)	21.27(21%)	1.1))- 1-3%

TABLE VII

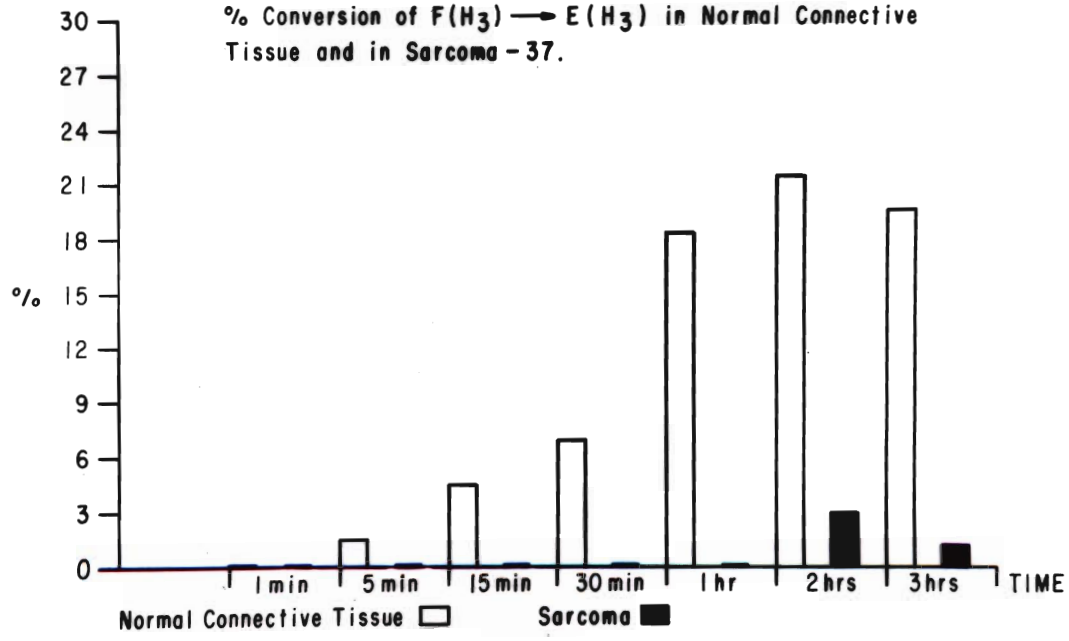
SUMMARY OF EXPERIMENTAL RESULTS
C^{14} PRODUCTS

NORMAL TISSUE

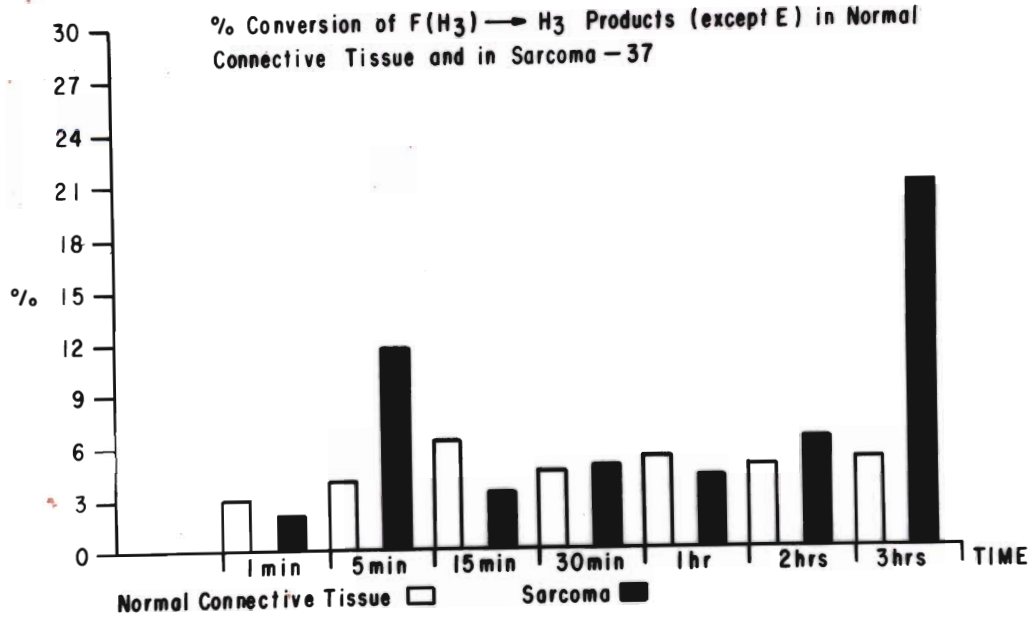
Time	R. "E"	R. "U"	C-19 Met. + Dr.	All Except F	E to F
1 min.	1.6	0.5	6.5	8.6	0
5 min.	2.3	0.8	5.7	8.8	0
15 min.	1.7	1.2	6.5	9.4	2.6
30 min.	1.8	1.1	5.9	8.8	4.4
1 hr.	2.4	2.0	6.7	11.1	9.2
2 hrs.	1.8	2.8	7.3	11.9	10.2
3 hrs.	2.1	4.6	6.1(6%)	12.8(12-13%)	40.0(40%)

SARCOMA TISSUE

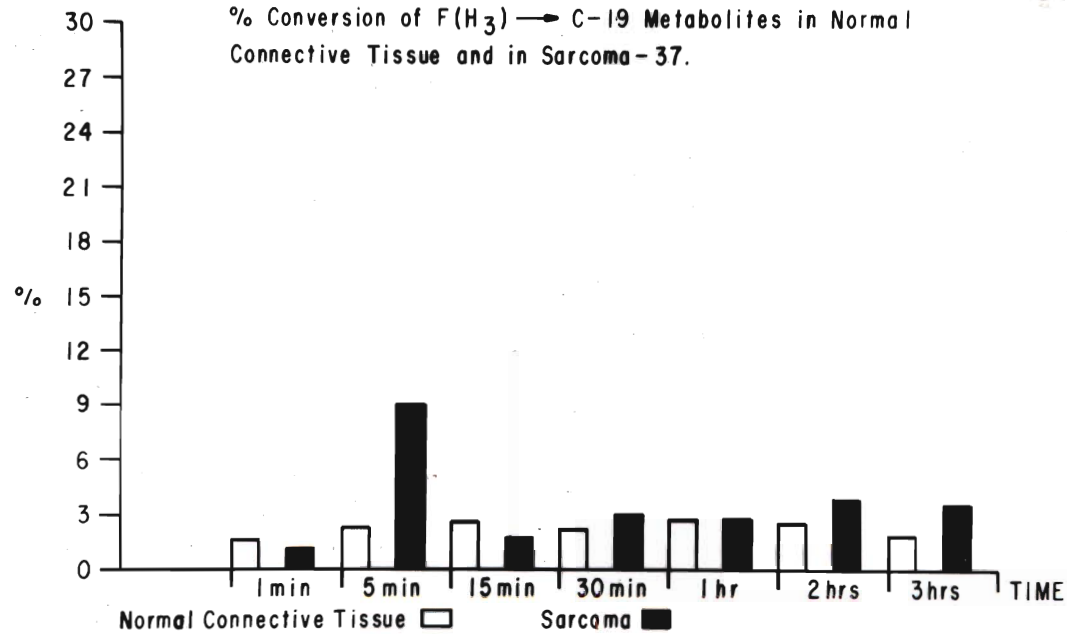
1 min.	0.36	9.2	4.1	13.7	0
5 min.	2.4	0.5	37.7	40.6	0
15 min.	1.8	1.2	23.6	26.6	0
30 min.	2.4	0.8	23.1	26.3	0
1 hr.	1.6	1.97	23.5	27.0	1.8
2 hrs.	1.7	14.3	10.7	26.7	3.7
3 hrs.	0.8	2.7	20.3(20%)	23.8(24%)	4.4(4%)


% Conversion of F(H3) → E(H3) in Normal Connective Tissue and in Sarcoma - 37.
30
27
24
21
18
%
15
12
9
6
3
0
1 min
5 min
15 min
30 min
1 hr
2 hrs
3 hrs
TIME
Normal Connective Tissue
Sarcoma

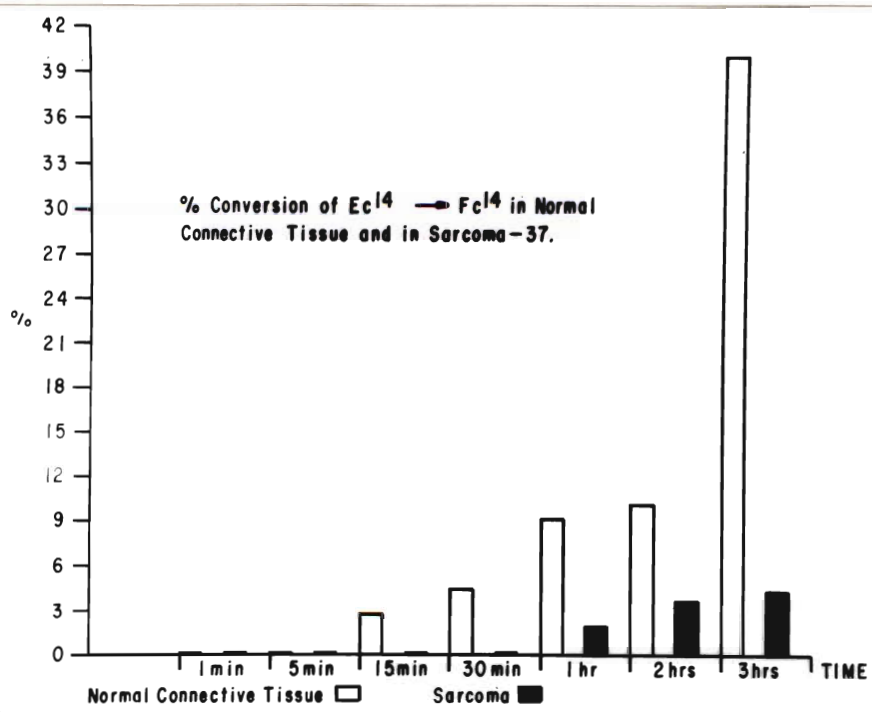
(5)


% Conversion of F(H3) → H3 Products (except E) in Normal Connective Tissue and in Sarcoma - 37
30
27
24
21
18
%
15
12
9
6
3
0
1 min
5 min
15 min
30 min
1 hr
2 hrs
3 hrs
TIME
Normal Connective Tissue
Sarcoma

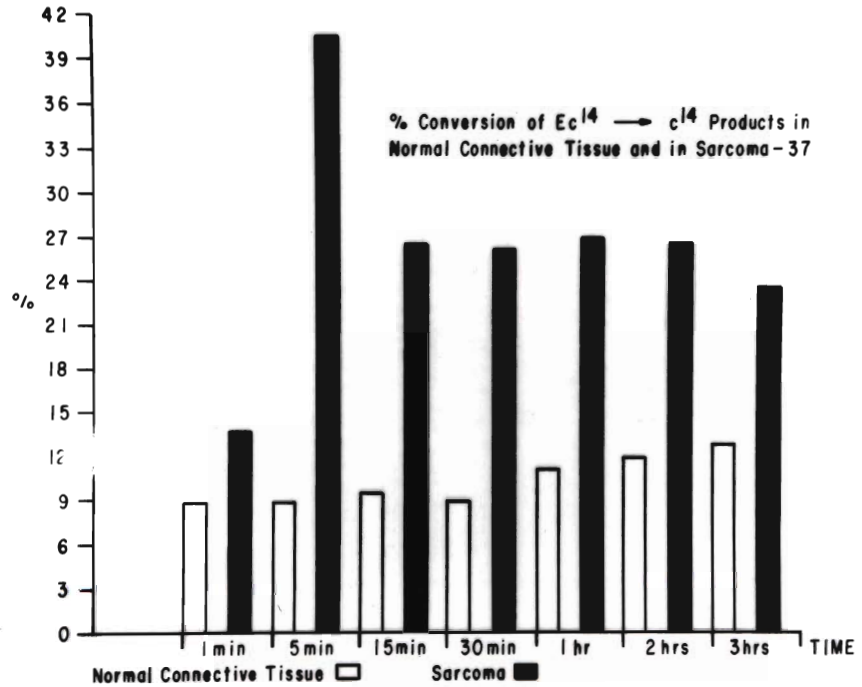
(6)


% Conversion of F(H3) → C-19 Metabolites in Normal Connective Tissue and in Sarcoma-37.
30
27
24
21
18
%
15
12
9
6
3
0
1 min
5 min
15 min
30 min
1 hr
2 hrs
3 hrs
TIME
Normal Connective Tissue
Sarcoma

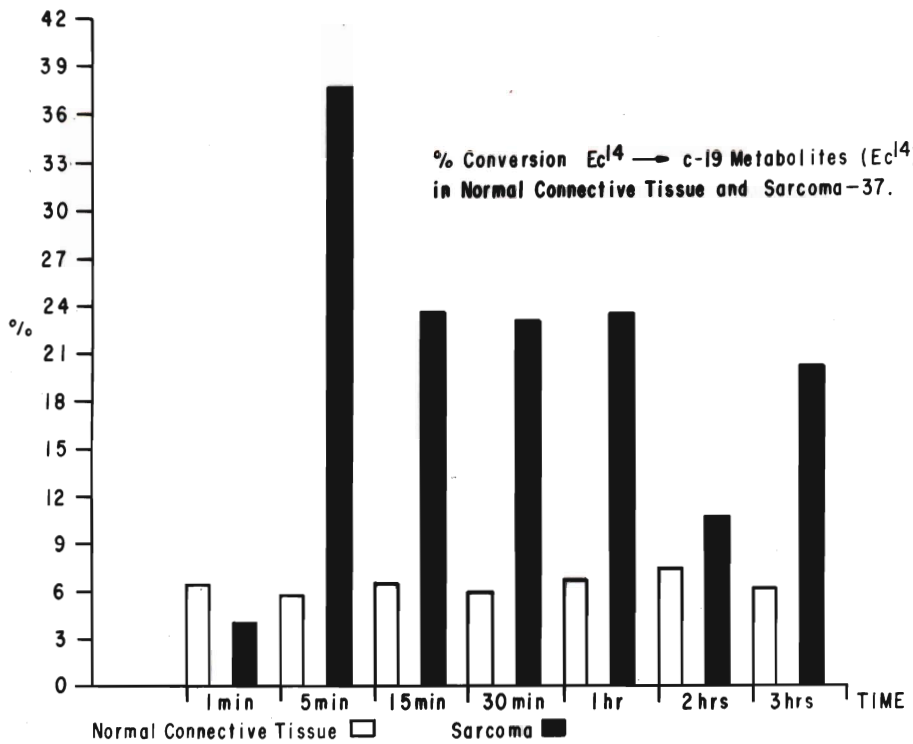
(7)


% Conversion of Ec14 → Fc14 in Normal Connective Tissue and in Sarcoma-37.
42
39
36
33
30
27
24
%
21
18
15
12
9
6
3
0
1 min
5 min
15 min
30 min
1 hr
2 hrs
3 hrs
TIME
Normal Connective Tissue
Sarcoma

(8)


% Conversion of Ec14 → c14 Products in
Normal Connective Tissue and in Sarcoma-37
%
42
39
36
33
30
27
24
21
18
15
12
9
6
3
0
1 min
5 min
15 min
30 min
1 hr
2 hrs
3 hrs
TIME
Normal Connective Tissue
Sarcoma

(9)


% Conversion Ec14 → c-19 Metabolites (Ec14)
in Normal Connective Tissue and Sarcoma-37.
%
42
39
36
33
30
27
24
21
18
15
12
9
6
3
0
1 min
5 min
15 min
30 min
1 hr
2 hrs
3 hrs
TIME
Normal Connective Tissue
Sarcoma

(10)

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VITA

Margaret Viko Coombs was born on April 28, 1928, in Salt Lake City, Utah. She attended Wasatch Elementary School and Bryant Junior High School in that city. Upon graduating from East High School in June, 1945, she received the Bausch and Lomb Honorary Science Award. She then entered the University of Utah, was a member of Pi Beta Phi Sorority and a Spur. After completing two years of medical school, she graduated from the University of Utah with honors in June, 1949, with a Bachelor of Arts degree.

She married Jack R. Coombs of Salt Lake City in 1949 and remained at home as a housewife and mother for the next several years.

In 1961, she returned to the University of Utah and began working toward her Master's degree in Anatomy.

She and her husband have three children: Kathy (16), Becky (13), and Michael (11).